CAUDAL CIRCULATION IN THE SHORT-FINNED EEL *ANGUILLA AUSTRALIS SCHMIDTII* (Phillips)

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ABSTRACT

Caudal Circulation in the Short-finned Eel *Anguilla australis schmidtii* (Phillips). P.S. Davie. Department of Zoology, University of Canterbury, Christchurch, New Zealand.

The anatomy, neuroanatomy and neural control of the caudal lymph heart of the short-finned eel are described. The evolution of accessory caudal vascular pumps is discussed.

An isolated saline perfused eel tail preparation is described which is suitable for investigation of vascular and interstitial fluid circulation. Dose versus vascular resistance response curves for adrenaline, noradrenaline and isoprenaline are presented. The eel tail vascular bed has alpha constrictory and beta two dilatory adrenergic receptors. Beta two receptors may be stimulated *in vivo* by sympathetic nervous activity. Vascular volume changes in the eel tail during constriction and dilation were small; less than three per cent of the tail vascular volume. Significant amounts of perfusate were extravasated when resistance increased more than ~ 100% of baseline resistance.

Frequency and amplitude of the lymph heart of the perfused tail increased when resistance increased by more than ~ 100% of baseline resistance. Frequency increases were probably caused by interstitial fluid build up rather than direct actions of vasoconstrictory drugs. One important function of the lymph heart is recovery of interstitial fluid from the tail.

Cardiac output, and ventral aortic, dorsal aortic and caudal venous blood pressures were recorded from unrestrained eels at rest, while swimming at 15 $cm.s^{-1}$ and 22-25 $cm.s^{-1}$, and after exercise. During swimming cardiac output fell slightly and transbranchial pressure differential increased. Elevated ventral aortic pressure was the principal cause of the increase in transbranchial pressure differential. Plasma catecholamine concentrations probably do not increase during swimming in the short-finned eel. Spontaneous cessation of swimming often occurred at very high branchial resistances. It is suggested that this response has a protective function for the gill tissues.

GENERAL INTRODUCTION

The series of reviews on topics in fish physiology edited by W.S. Hoar and D.J. Randall (1970) and the monograph 'Circulation in Fishes' by G.H. Satchell (1971) have stimulated research into many areas of fish physiology. Fish species which are important food sources have provided most of the information on which our understanding of fish cardiovascular physiology is based. Although our knowledge of the cardiovascular system in species of salmonid, eel and cod is relatively comprehensive, there remain several important problems to be resolved such as whether blood flow to vascular beds is controlled by circulating catecholamine concentrations or by sympathetic nervous activity. Questions such as the role of the lymphatic circulation in fishes have still to be asked.

This thesis is mainly concerned with two aspects of circulation of body fluids within the tail of the short-finned eel (*Anguilla australis schmidtii*). These are the control of vascular resistance of the eel tail and the function and functioning of the caudal lymphatic heart. The text is divided into three sections each of which begins with a full introduction and ends with a summary of the principal findings and conclusions. The first section is an account of the caudal lymphatic heart as an organ and describes the anatomy and neural control system of the lymph heart. The second deals with the vascular bed of the eel tail in isolation from the rest of the body, particularly its responses to sympathetomimetic drugs. The third section is a study of the intact eel circulatory system during swimming in an attempt to relate isolated tissue results to the animal as a whole.

PART I THE CAUDAL LYMPHATIC HEART OF *ANGUILLA AUSTRALIS SCHMIDTII*

I.1 Introductions

I.1.1 The lymphatic system of fishes

Lymphatic vessels are an integral part of the circulatory system of vertebrates. Embryologically lymph vessels are derived from mesenchyme and are phylogenetically almost as old as the veins and arteries of chordates. Cephalochordates have sacs or spaces equivalent to the lymphatics of higher vertebrates, but the first appearance of true lymphatics occurs within the fishes (Kampmeier, 1969).

Lymphatics are divisible into capillaries, precollecting ducts and collecting ducts on the basis of size and the structure of the vessel wall. Lymphatic capillaries are the smallest, with a diameter of ~ 10-50 μm. The walls are composed of very permeable endothelium only (Casley-Smith and Florey, 1961). Lymph capillaries are not continuous with the arterio-venous system, but end in blind sacs within tissues. Precollecting lymph ducts have diameters that vary between ~ 20 and 200 μm. These ducts have a layer of elastic connective tissue surrounding the endothelium and carry lymphatic fluid from the lymphatic capillaries through the tissue bulk to large collecting ducts. The larger collecting ducts have diameters greater than ~ 200 μm. Their walls have layers of smooth muscle surrounding the endothelium and elastic connective sheaths. In mammals large collecting ducts have valves which prevent backflow of lymph. The largest of lymph vessels in mammals, the thoracic duct, empties into the innominate vein through a valved orifice thereby returning lymph to the blood vascular system (Battezzati and Donini, 1972).

The primary function of lymphatics is to recover interstitial fluids and solutes, particularly proteins from tissues. In higher vertebrates the lymphatic system has assumed a number of other functions including haemopoesis, anti-infection action by lymphocytes and antibodies, glandular secretions and fat transport within the chylous vessels.

The formation of lymph has received a great deal of attention. The question of how lymph is formed can only be satisfactorily answered once an understanding of the dynamic equilibrium that exists between blood tissue and lymph has been gained. Starling (1894, 1896) proposed

a theory which later became known as the Starling hypothesis. This hypothesis describes the transcapillary exchange processes; it basically states that the efflux of fluid into tissue from the arterial end of capillaries containing oxygen, ions and nutrients is proportional to the fluid hydrostatic pressure within the capillaries. This is the process of ultrafiltration or convection. Hydrostatic pressure decreases over the length of the capillaries as a result of fluid loss and friction against vessel walls. At the venous end of capillaries, low hydrostatic pressure and a high colloid osmotic pressure, because of restricted movement of very large molecules through the capillary walls, causes resorbtion of fluid lost to the tissues at the arterial end. Thus by constant efflux of fluid at the arterial end and resorbtion at the venous end tissues adjacent to capillaries are continuously bathed with fluid derived from the blood. The hypothesis still stands as the basis for our current concepts of capillary exchange processes with additions and modifications from subsequent research (see Pappenheimer and Soto-Revera, 1948; Mellander and Johansson, 1968; Barcroft, 1976; Mellander, 1978).

Lymph is derived from interstitial fluid. Thus lymph formation can be described by three separate but related mechanisms. First the production of tissue fluid by ultrafiltration, diffusion and vesicular exchange across the 'arterial' capillary walls. Second, interstitial fluid moves through the interstitium transporting and exchanging solutes. Third, tissue fluid either leaves the interstitium via the 'venous' capillaries or is sequestered into the blind endings of the lymphatic capillaries which penetrate into almost all tissues.

Tissue fluid does not exist as a free solution but is bound onto hydrophyllic connective tissue fibres that form the matrix between the cells. Movements of water and molecules through this space is by restricted diffusion. Large molecules (> 60 nm in diameter) behave as if travelling through a 'gel column' rather than through a fluid space (Watson and Grodinis, 1978).

Sequestration of fluid from the matrix into the very porous lymphatic endings remains largely unexplained. It was considered that the positive pressure differential between tissue and lymphatic fluid of ~ 0.05 kPa was the driving force (see Barcroft, 1976). Whether or not the tissue fluid exerts a positive or negative hydrostatic pressure is uncertain (see McMaster, 1946; Guyton, Granger and Taylor, 1971). Other mechanisms suggested include active transport, phagocytosis and changes in the bonds between water and connective fibres which could allow free solutions to form near the lymphatic endings. This last

hypothesis was advanced by Mottura (1960) when he stated that,

> "... the lymphatic channel system originates where the characteristic linear macromolecular structures of the ground substance cease. At this point the absorbtion capabilities of the ground substance change and free fluid is able to form. The ground substance changes its cellular conformation from matrix to endothelial sheets which gradually take on the characteristic features of the lymphatic capillaries."

Although the details of lymph formation remain obscure, a wealth of evidence from isolated prefused preparations (see Starling, 1894; McMaster, 1946; Arturson, Groth and Grotte 1972; Brace and Guyton, 1977) clearly shows that when capillary blood pressure is raised, interstitial fluid production increases. With greater tissue fluid volume, and possibly pressure, lymph production increases until a new equilibrium is established at the blood capillaries. If elevated blood capillary pressures are maintained, resorbtion at the 'venous' capillaries and ultrafiltration at the 'arterial' capillaries reach a new equilibrium and lymph production declines toward pre-perturbation rates.

The lymphatic anatomies of more species of fish have been described than any other animal class (Kampmeier, 1969). Within the fishes there is a transition from anatomically poorly defined lymph systems to anatomically discrete, functionally competent lymphatics (Casley-Smith, 1971).

Of the Agnatha, the petromyzonts have been best described. They have extensive well defined lymphatic vessels and sinuses in addition to the arterio-venous system. Many of the early descriptions relied upon injection methods to visualise the vasculature. The valves that guard lymphatico-venous connections in petromyzonts are weak, consequently blood or injected solutions were often forced back from the veins into the lymphatic vessels (see Johansen, Fange and Johanneson, 1962). Thus many lymphatic vessels were originally designated as veins. Most of these problems have been resolved and Kampmeier's descriptions appear to have taken account of inaccuracies in early descriptions.

The elasmobranchs (Chondrichthyes) are believed to have evolved in parallel with the bony fish (Osteichthyes). However the elasmobranch lymphatic system appears degenerate and the species described show the lymphatic system to be poorly defined and greatly reduced compared to other fishes. Again the problems of differentiation between veins and lymphatic vessels arose. In many elasmobranchs veins are present in the

positions where lymphatic vessels are found in other fishes. For example the lateral cutaneous vein in the dogfish occupies the same position as the lateral cutaneous lymphatic vessels of teleosts. Furthermore, in this vein the blood flows caudally toward the tail venous pump (Birch, Carre and Satchell, 1969). Elasmobranchs have only two distinct lymphatic vessels, the cardial lymphatics, which are very small.

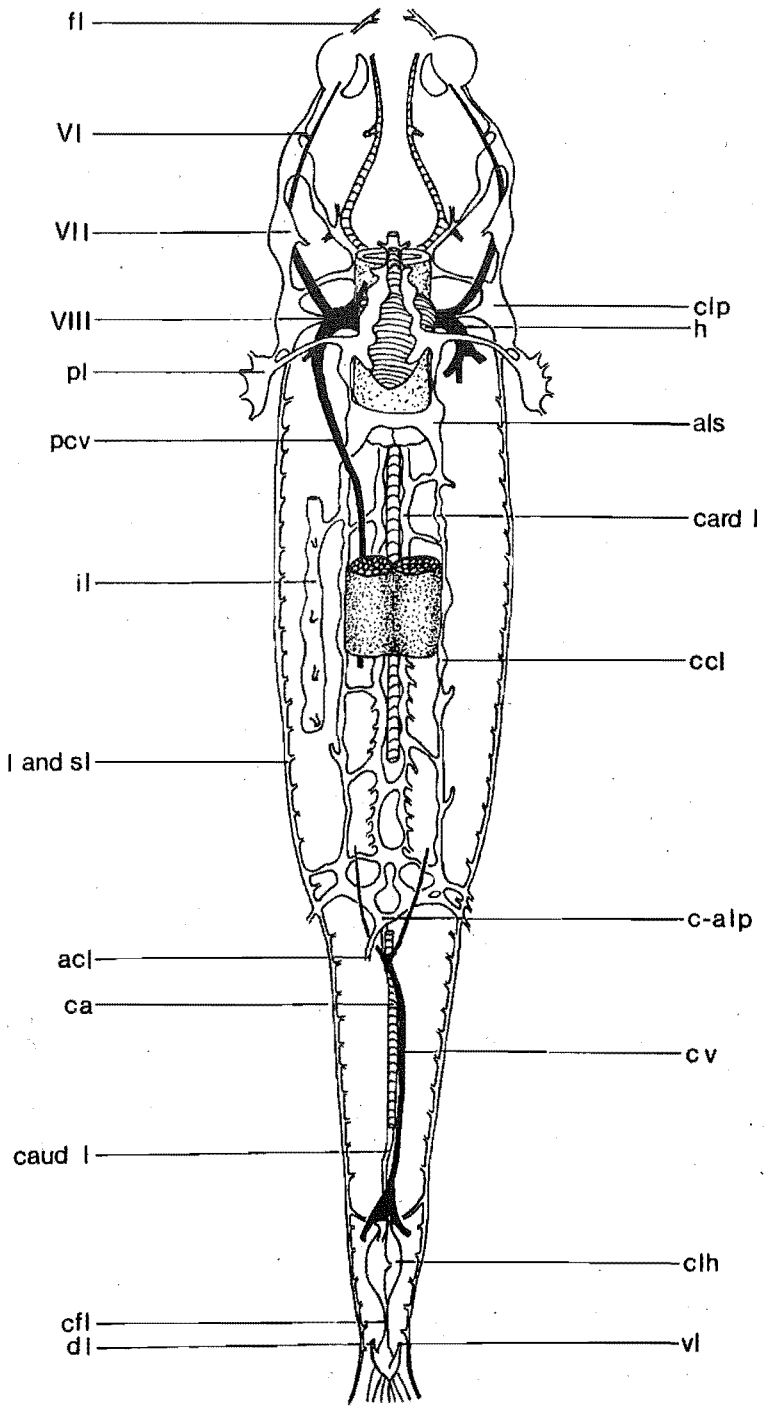
Within the Osteichthyes, the Chondrostei have a lymphatic system which is similar in position and extent to that of the elasmobranchs (Favaro, 1906; Allen, 1910). Alternatively the Holostei, or bony ganoids, and the Teleostei have extensive and well defined lymphatics. A generalized composite diagram of the teleost lymphatic system is presented in figure I.1. Three lymphatico-venous connections commonly occur within the head of teleosts, (vents I, II, III) while a single connection is observed at the tip of the tail. There is considerable variation between species and indeed between individuals within the same species.

I.1.2 Accessory vascular pumps

At the lymphatico-venous junctions, the lymphatic sinuses often possess the ability to propel their contents into the blood stream. Where the propulsion is provided by adjacent muscles whose prime function is other than lymph propulsion, these are designated lymph propulsors, after Allen (1910). Where the lymph sinus is associated with muscles whose principal function is to pump lymph, they are called lymph hearts. Rostral and caudal lymph propulsors are found in most teleost orders. Agnatha have a unique caudal blood propulsor which was described by Greene (1899) and Allen (1917) in the hagfish *Polistotrema stouti* and by Johansen (1963) in *Myxine glutinosa*. *Mustelus antarcticus* has no lymph propulsors but possesses a caudal venous blood pump which in some ways resembles the caudal lymph hearts of teleosts (Satchell, 1971).

Leeuwenhoek (1695, cited in Polimanti, 1912) first described the lymph heart of the eel as a "Höhlen-und Klappenapparat", or hollow beating organ. Since this initial observation four reviews of fish lymph hearts have been published (Favaro, 1906; Polimanti, 1912; Przemyska-Smosarska, 1951; Kampmeier, 1969). Both Polimanti and Przemyska-Smosarska failed to cite all the available information. Favaro provided an exhaustive review of the topic which has been brought up to date and reduced in size by Kampmeier (1969). These publications concentrated upon anatomy and the occurrence or absence of the lymph heart in various taxa. Caudal lymph hearts are common in most teleost

FIGURE I.1 Generalised lymphatic vascular anatomy of teleosts. a.c.l., accessory caudal lymphatic; a.l.s., abdominal lymphatic sinus; c.a., caudal artery; c-a.l.p., circum-anal lymphatic plexus; c.cl., collateral cardial lymphatics; card.l., cardinal lymphatic; caud.l., caudal lymphatic; c.f.l., caudal fin lymphatic; c.l.h., caudal lymph heart; c.l.p., cervical lymph propulsor; c.v., caudal vein; d.l., dorsal lymphatic; f.l., facial lymphatic; h., heart; i.l., intestinal lymphatic; l. and s.l., lateral and segmental lymphatics; p.c.v., posterior cardial vein; p.l., pectoral lymphatic; VI, VII, VIII, vents I, II, III. (After Kampmeier, 1969).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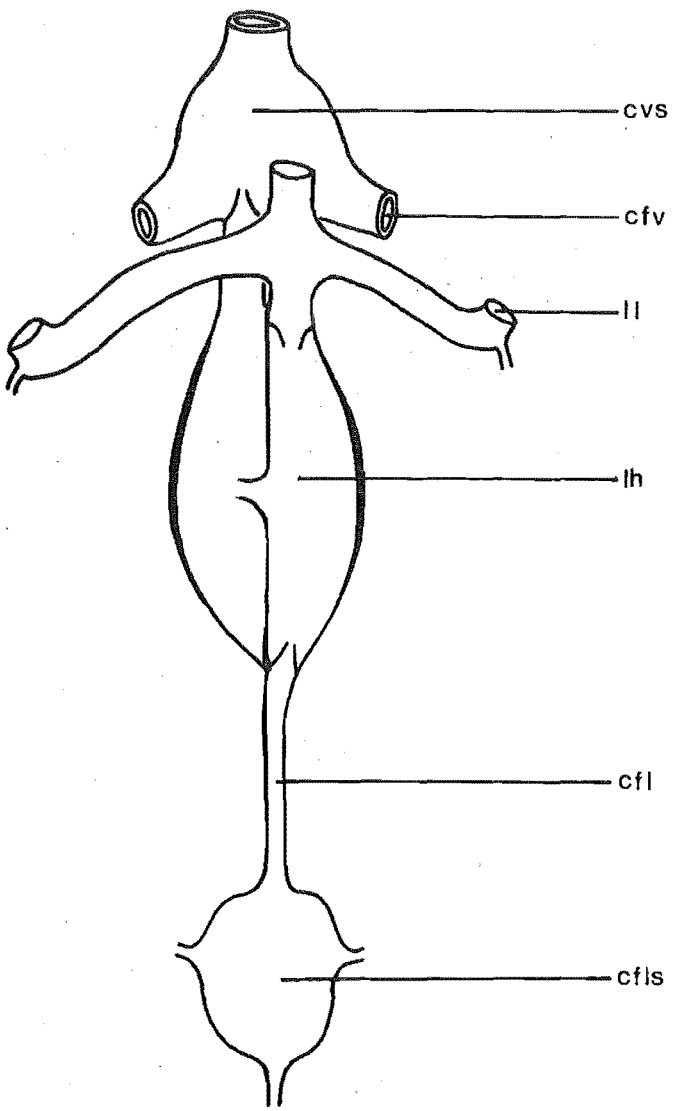
orders, except the Acanthopterygii, the most advanced order. Within the teleosts there is evidence for the evolutionary development of both lymph vessels and lymph hearts. The most complex lymph heart is found in the Anguillidae.

Lymph hearts are present in other vertebrate classes. Anurans have two coccygeal and two cervical lymph hearts (see Schipp and Flindt, 1968). Tailed amphibians have a series of smaller lymph hearts arranged segmentally along the length of the tail. Kampmeier (1969) stated that all reptiles so far examined have lymph hearts, often segmentally arranged along the length of the body. Bird embryos occasionally exhibit a caudal lymph heart which is lost after reaching maturity (Sala, 1900). Although no data are available for Monotremes, lymph hearts are absent in Eutheria and Metatheria. Large mammalian lymph collecting ducts have regularly contracting valved vessels which assist lymph flow (Mislin and Rathenow, 1962; Hargens and Zwefach, 1977). Mammalian jejunal lacteals appear to be microscopic lymph pumps (Lee, 1974).

The mechanism by which lymph is propelled from lymph hearts varies between vertebrate classes. As mentioned before lymph propulsors derive propulsive force from the surrounding muscle. True lymph hearts have specific muscles assigned to this role. Favaro (1905) described the pattern of flow through the lymphatics in the area of the lymph heart of the european eel which is presented in figure I.2. Lymph is received by the right chamber from the caudal, caudal fin and lateral lymphatics. Lymph is then pumped from the right side through the hypural foramen to the left side from where it is expelled into the caudal vein through a short extension of the left side of the lymph heart. Control of the muscular contractions of the lymph heart was studied by Mislin (1960). He concluded from experiments exposing different parts of the body to different temperatures that frequency was controlled by, or at least influenced by higher central nervous centres, despite many early records of maintained contraction after spinal cord destruction (see Polimanti, 1912). Chan (1971) suggested on the basis of pharmacological evidence that the lymph heart was myogenic. However no clear account of how lymph heart beat is maintained or modified has been published.

Anuran lymph heart muscle receives excitatory nicotinic and inhibitory muscarinic cholinergic innervation from the XIth spinal nerve (Brüke and Umrath, 1930, 1933; Obara, 1962; Day, Rech and Robb, 1963; Such, 1968). Mammalian lymph vessels are constricted by smooth muscle which from their pharmacology appear to be controlled in a similar

FIGURE I.2 Circulation pattern through the caudal lymphatic vessels of the european eel. c.f.l., caudal fin lymphatic; c.f.l.s., caudal fin lymphatic sinus; c.f.v., caudal fin vein; c.v.s., caudal venous sinus; l.h., lymph heart; l.l., lateral lymphatic. (After Favaro, 1905).


cvs
cfv
ll
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manner to blood vessels (Ohhashi, Kawai and Azuma, 1978).

The eel lymph heart was chosen for this study of the lymph heart because it is well described and is the most advanced example of teleost lymph hearts. Despite the long history of research on eel lymph hearts, information is lacking in several areas. The gross anatomy is well established but fine detail of the lymph heart muscle has not been described. Apart from the preliminary study by Davie (1975) there are no reports on teleost lymph heart neuroanatomy. Thus questions as to the number of nerves and axons that innervate the lymph heart, the types of myoneural junctions and the presence and type of sensory structures need to be answered. The aims of this section are to confirm previous anatomical studies and to provide the answers to the above questions at the light and electron microscope levels. After the neuroanatomy is established a study of the control system of the eel lymph heart is made.

I.2 Experimental Animals

Short-finned eels (*Anguilla australis schmidtii* Phillips) used in this study were trapped with fyke nets in the Selwyn River near the 'Selwyn Huts' (45°43'S, 172°26'E). The long-finned eel (*Anguilla dieffenbachi* Gray) was also trapped but was less common. The ecology of the short-finned eel has recently been described by Ryan (1978). Eels were maintained for at least two weeks after capture in an indoor tank (volume 1600 *l*), under conditions of low illumination. Experiments were performed within six weeks of capture during which time fish were not fed. In none of the eels was there signs of silvering, the preparation for migration. The Selwyn River flows into Lake Ellesmere where New Zealand's most important eel fishery is sited. Commerical fishing in the Selwyn River however is not permitted.

I.3 Anatomical Methods

I.3.1 Gross anatomy

The vascular anatomy of the region of the lymph heart is described with the vascular anatomy of the whole tail, in section II.2.

Skeletal elements of the tail were exposed by boiling the caudal-most part of the tail in 10% KOH, or by digesting away the tissue in a solution of 0.22% pancreatin and 1.75% KOH at 37°C. The bones left were stained in 1% alizarin red until they appeared red. The preparations were dehydrated in ethanol and mounted under glass in balsam.

I.3.2 Microscopic techniques

I.3.2.1 Fixation

Blocks of tissue no thicker than three mm were fixed in either aged alcoholic Bouins fluid (Gregory, 1970) for 24-48 hours, 10% neutral buffered formaldehyde (Lillee, 1965) for three weeks, or occasionally in 8% formic acid-butanol-propanol solution (Blest and Davie, 1978) for 60 minutes. These blocks were decalcified in 5% trichloroacetic acid for 8-12 hours before dehydration. After dehydration the blocks were cleared in either terpineol or benzene before embedding in three changes of fresh paraffin wax (56°C).

I.3.2.2 Sectioning

Sections were cut on a Beckman microtome at 8 µm unless otherwise stated and affixed onto glass slides with 1 : 1 glycerine-albumin mixture. This adhesive was vacuum filtered through a sintered glass and diluted 20 times with distilled water and used as a floatation medium.

I.3.2.3 Frozen sections

Material to be used for motor end plate cholinesterase staining methods was fixed for 12 hours in neutral buffered paraformaldehyde (McIsaac and Kiernan, 1974). Frozen sections were cut at between 20 µm and 40 µm on a Brights freezing microtome, (Huntingdon, England) and collected onto distilled water.

I.3.3 Staining

I.3.3.1 Methylene Blue

The last three to five vertebral segments were removed from

decapitated animals. The skin and overlying muscles were cleared from the lymph heart area and the tissue left overnight in 0.03% methylene blue in fresh water eel ringer at 4°C. These preparations were examined fresh or fixed in saturated ammonium molybdate solution for two hours. Fixed preparations were dehydrated in ethanol series and stored in cedarwood oil.

I.3.3.2 Modified Holmes-Blest reduced silver stain (after Blest, 1961)

1. Fix in aged Dubosq-Brasil (Gregory, 1970)
2. Clear in terpineol
3. Wax embed
4. Impregnate with 20% $AgNO_3$ - 2½ hours
5. Rinse in distilled water - 5 minutes
6. Incubate in the following solution - 18 hours at 37°C

1/5 M boric acid	27.5 ml
1/20 M borax	22.5 ml
1% $AgNO_3$	3.0 ml
Pyridine	2.0 ml
2,6-lutidine	2.0 ml
distilled water	250 ml

   pH is 8.4 at 20°C, measured with B.D.H. narrow range indicator strips
7. Reduce in the following solution for 3 minutes at 55-60°C

Hydroquinone	1 g
Anhydrous $Na_2S_2O_3$	10 g
Distilled water	300 ml
8. Wash in tap water - 3-5 minutes
9. Rinse in distilled water
10. Tone in 0.2% $NaAuCl_4.2H_2O$ - 1-3 minutes
11. Rinse in distilled water - 1 minute
12. Reduce in 2% oxalic acid - 5 minutes
13. Wash in distilled water - 3 minutes
14. Fix in 5% $NaSO_3$ - 1½ minutes
15. Wash in distilled water thoroughly - 5-10 minutes
16. Dehydrate and mount

Times for each of the stages 4, 6 and 10 were varied to obtain the best results. Stages 11 to 13 were repeated until observation of the sections revealed that sufficient toning had occurred. The length of time needed to adequately tone these preparations was between 75

and 180 seconds.

I.3.3.3 Masson's trichrome stain (after Pantin, 1948)

1. Fix in aged Dubosq-Basil (Gregory, 1970)
2. Wax embed
3. Stain in Weigerts - 3 minutes
4. Wash in tap water - 15 minutes
5. Stain in Bieliech Scarlet till darker than finally required - 30 seconds
6. Rinse in distilled water
7. Differentiate in phosphomolybdic acid - 4 minutes
8. Stain collagen in Fast green - 30 seconds
9. Dehydrate rapidly, i.e. into 90% ethanol then to 100% and mount.

I.3.3.4 Bromoindigo end plate stain (after McIsaac and Kiernan, 1974)

1. Free floating sections are incubated in the following solution for 45 minutes at 37°C.

5-Bromoindoxyl acetate (Sigma)	1.3 mg
Ethanol to dissolve	0.1 ml
0.05 M Potassium ferrocyanide	1.0 ml
0.05 M Potassium ferricyanide	1.0 ml
Tris - HCl buffer pH 7.2 (Pearse, 1968)	2.0 ml
0.1 M $CaCl_2$	1.0 ml
Distilled water	to 10.0 ml

   Sections were handled with glass instruments at all times before incubation.
2. Rinse in distilled water
3. Mount on glass slides with glycerine-albumin adhesive and dried for 60 minutes

At this stage the sections were either dehydrated and covered or taken through the Modified Holmes-Blest reduced silver stain. Cholinesterase staining with the thiolacetic acid method (Nakata and Nishijima, 1971) proved unsuccessful on this tissue.

I.3.3.5 Electron microscope grid preparation

1. Fix in glutaraldehyde fixative - 1 hour

25% Glutaraldehyde	5.0 ml
0.2 M Cacodylate buffer	12.5 ml

1.0 M sucrose 5.0 ml

Make up to 50 ml with distilled water

2. Wash in washing solution - ½ hour

   Quantities as above but omit glutaraldehyde and increase 1.0 ml sucrose to 15 ml

3. Post fix in 1% Osmium tetroxide - 1½ hours

   0.1 g OsO_4 in 10 ml of washing solution

   store at 4°C

4. Gently agitate in two changes of propylene oxide for ¼ hour each

5. Transfer tissue to 1:1 mixture of resin and propylene oxide and gently mix for longer than 2 hours

6. Then transfer to 9:1 resin : propylene oxide for longer than 12 hours - 24 hours

7. Place tissue in casting vials with fresh resin and leave for 2 days at room temperature

8. Polymerise at 60°C for 24 hours

Spurr's Resin (after Spurr, 1969)

(i)	E.R.L. 4206	3.33 g
(ii)	D.E.R. 736	2.00 g
(iii)	N.S.A.	8.66 g
(iv)	S-1 (hardener)	0.12 g

The first three ingredients (i-iii) are mixed gently together before the addition of the hardener (iv).

I.4 Anatomical Results

I.4.1 Introduction

Favaro (1905, 1906) described in detail the anatomy of the lymph heart of many species of fish including the european eel. Kampmeier (1969) summarised these findings, adding more recent findings of Hoyer (see Hoyer, 1934, cited in Kampmeier, 1969). The gross anatomy of the lymph heart of this species closely resembles that of the short-finned eel. A preliminary report of these results was presented in an unpublished undergraduate project (Davie, 1975).

I.4.2 Caudal skeleton

Figure I.3 is a diagram of the skeletal elements in the caudal most centimetre of the short-finned eel tail. The last vertebra is modified caudally to form two flattened plates, the hypural plates (h). The gap between the hypural plates is the hypural foramen (h.f). Just caudal of the hypural plates is the hypural canal (h.c). The haemal zygaphophysis (h.z) of the left side is laterally and rostrally extended. The urostyle (u) combines with the specialized neural processes (s.n.p) from the most posterior part of the neural arch (n.a) in which is found the urophysis, or caudal neurosecretory organ. The penultimate vertebra is unmodified from the typical teleost caudal vertebra (Parker and Haswell, 1962). The neural spine (n.s) forms the neural arch (n.a) dorsally and provides support for the neural arch processes (n.p) onto which the dorsal fin rays are attached. Ventrally the haemal spine and its processes form the haemal arch and support the ventral fin rays.

I.4.3 Lymph heart muscle

The lymph heart appears as two flattened oval pink bodies about two to three mm in length, lying either side of the hypural plates. It is readily observed when the skin and overlying tissue of the caudal-most part of the tail are dissected away. In fresh specimens the pink colour indicates the high blood vessel density in the muscles.

Transverse sections (see figure I.4a) show that the lymph heart lumen is lined with endothelium. A layer of connective tissue is interposed between endothelium and muscle tissue. Valves are observed in the hypural foramen although because of tissue hardness in the area valvular tissue was often fragmented and lost whilst sectioning. There

FIGURE I.3 Diagram of the skeletal components in the area of the last three vertebral segments of the tail. The lymph heart covers the shaded area. h., hypural plates; h.a., haemal arch; h.c., hypural canal; h.f., hypural foramen; h.s., haemal spine; h.p., haemal process; h.z., haemal zygapophysis; n.a., neural arch; n.p., neural process; n.s., neural spine; s.n.p., specialised neural process; u., urostyle; v.f.r., ventral fin rays.

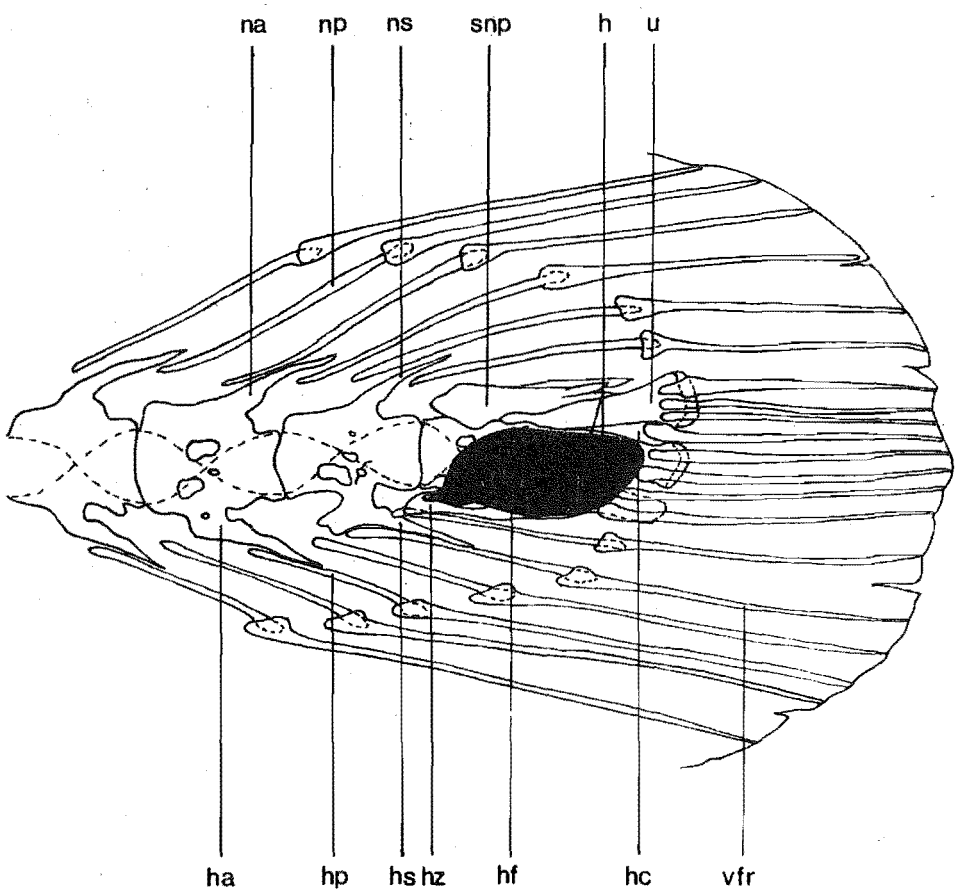
na
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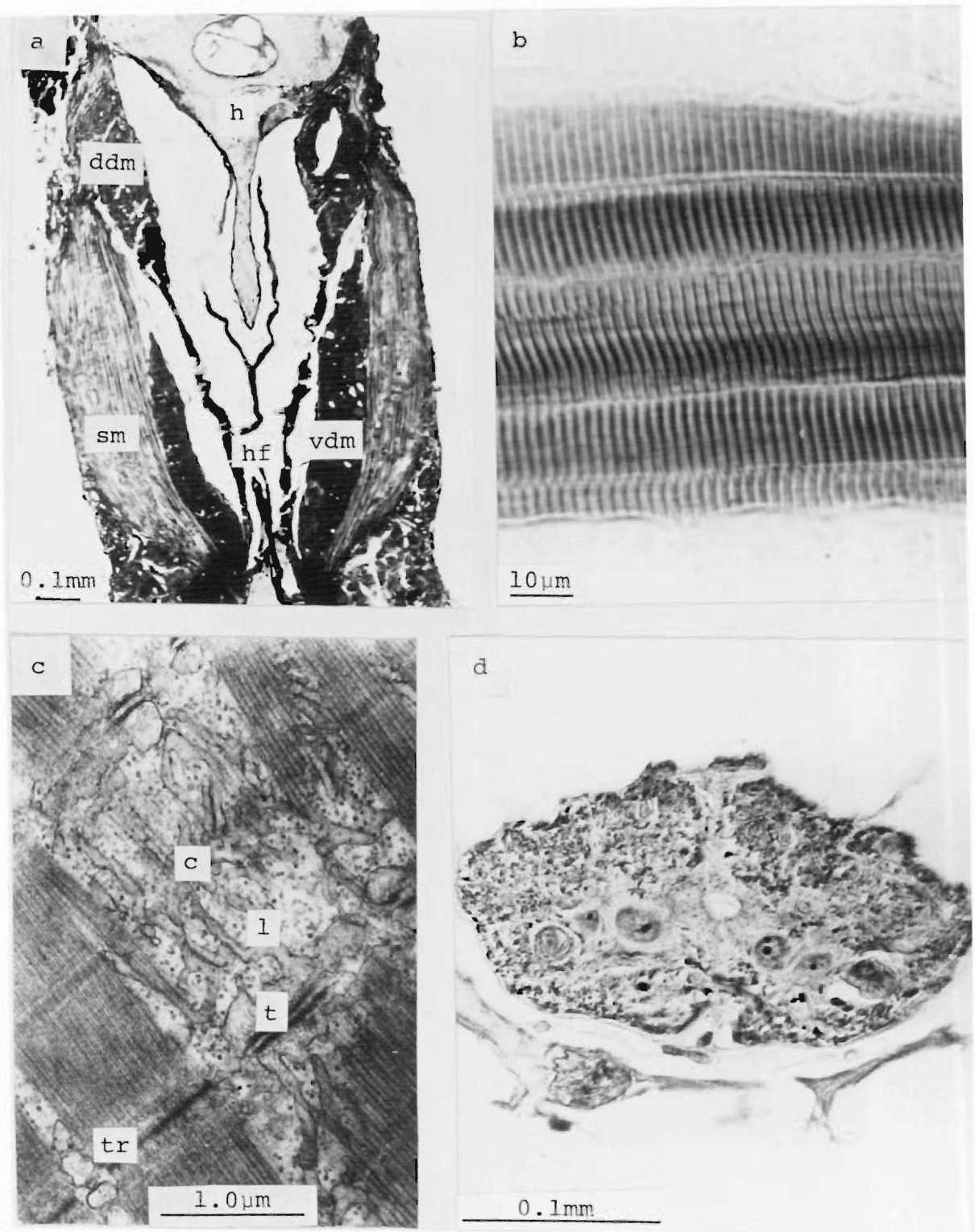
FIGURE I.4a Light micrograph of a transverse section through the short-finned eel caudal lymph heart. h., hypural plate; h.f., hypural foramen; d.d.m., dorsal deep muscle block; v.d.m., ventral deep muscle block; s.m., superficial muscle block. (Masson's trichrome).

FIGURE I.4b Light micrograph of lymph heart superficial muscle fibres. (Holmes-Blest reduced silver).

FIGURE I.4c Electron micrograph of lymph heart superficial muscle showing the extent of the sarcoreticular system. The system fuses near the Z line and in the centre of the A band to form a fenestrated network over the surface of the sarcomere. c., collar; l., longitudinal system; t., transverse system; tr., traids at the junction of the transverse and longitudinal systems.

FIGURE I.4d Light micrograph of transverse section of spinal cord from the penultimate vertebral segment. (Holmes-Blest reduced silver).


a
h
ddm
sm
hf
vdm
0.1mm
b
10µm
c
c
l
t
tr
1.0µm
d
0.1mm

are three muscle blocks on each side of the lymph heart. The outermost muscle block (superficial muscle, S.F.) has fibres which run dorso-ventrally. The two inner muscle blocks both have fibres in a rostro-caudal orientation. These muscles have been designated the dorsal and ventral deep muscle (D.D.M, V.D.M) respectively. This description is essentially the same as that of Favaro (1906) for the european eel.

At higher magnifications the muscle fibres in each of the three muscle blocks are seen to be striated with similar sarcomere lengths of around 2.0 µm (see figure I.4b). Nucleii are located peripherally and numerous small blood vessels extend between muscle fibres. At electron microscopic level (see figure I.4c) typical striated muscle structure is seen. Numerous glycogen granules are visible around the muscle cells and an extensive sarcoplasmic reticulum (S.R.) extends over the muscle surface. The junctions of the S.R. and the transverse tubule or T system occurs between adjacent cells at the Z line and forms distinctive structures called triads.

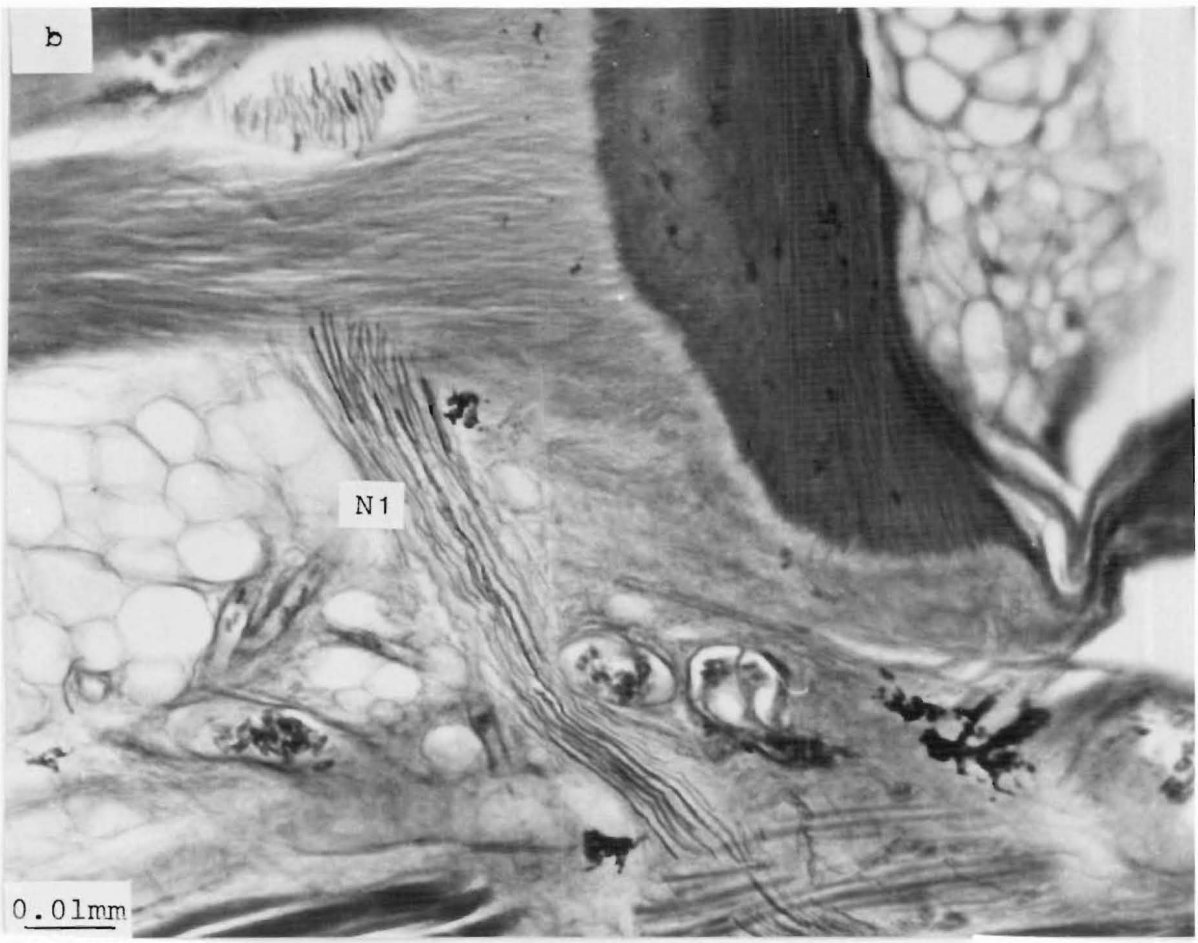
I.4.4 Nerves in the area of the lymph heart

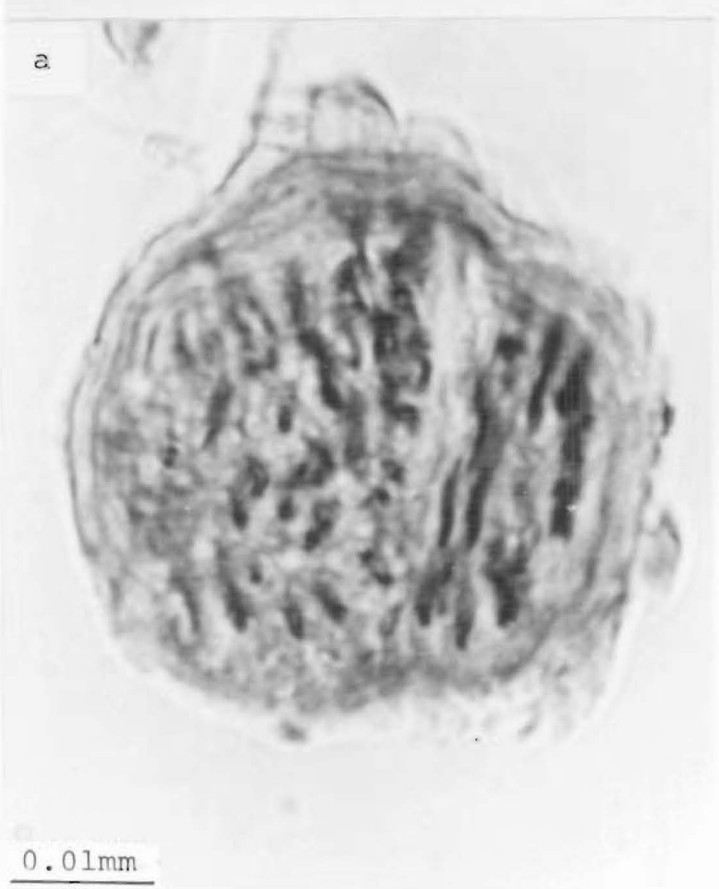
A reduced silver staining technique was chosen specifically to stain the nerves in the area of the lymph heart. Methylene blue preparations revealed little because the connective fibres, blood vessels and muscle fibres also took up the dye.

Transverse sections of the area and of the spinal cord alone showed that dorsal to, and toward the rostral end of the penultimate vertebra, dorsal and ventral nerve roots emerged (see figures I.4d and I.7). Within this area of the cord the anterior end of the urophysis is also evident, occupying the ventral part of the cord and extending caudally where it occupies the whole of the spinal cord. The ventral and dorsal roots converge within the neural arch and emerge from the vertebra through a single foramen. Some fibres from this tract enter a ganglion of cell bodies, possibly a sympathetic ganglion, located in a cavity half way up the outside of the vertebra. Most of the fibres pass through this point and traverse the vertebra ventrally. At a point lateral and adjacent to the haemal arch of the penultimate vertebra the nerve trunk turns caudally and merges with the nerve from the adjacent rostral vertebra (see figures I.5a, I.5b). This combined nerve trunk passes caudally for about 1 mm to the rostral tip of the lymph heart muscle where it divides. The nerve from the third to last vertebra runs dorso-caudally across the surface of the lymph heart superficial muscle and passes through the hypural canal to the posterior fin rays.

FIGURE I.5a Light micrograph of transverse section through lymph heart nerve (Nl). The two bundles of axons both contain myelinated and nonmyelinated axons which can be visualised by using different histological techniques. (Holmes-Blest reduced silver).

FIGURE I.5b Light micrograph of longitudinal section through the lymph heart nerve (Nl). The fibres traverse ventro-caudally from the spinal foramen for approximately 1 mm, then extend caudally toward the cephalic end of the lymph heart muscles. Nl, lymph heart nerve. (Holmes-Blest reduced silver).


b
N1
0.01mm


a
0.01mm

The nerve from the penultimate vertebra spreads over the surface of the lymph heart and subdivides. The same description applies to both sides of the lymph heart. Most nerve fibres extend up to the dorsal edge of the superficial muscle and small fibres extend ventrally from the dorsal edge.

The small fibres were traced to their endings in the muscle and were visualized with the aid of the bromoindigo cholinesterase stain (see figure I.6a). The end plates are small (5-12 μm) 'en plaque' type (Bone, 1964). Electron microscope preparations of the end plates show uncharacteristic absence of extensive interdigitations at the myoneural junctions. The spherical vesicles within the terminal are 50-60 nm in diameter and agranular, suggesting cholinergic vesicles (Uehara, Campbell and Burnstock, 1976) (see figure I.6b).

Figure I.7 is a composite diagram giving the neuroanatomy of the lymph heart of the short-finned eel. Spinal nerve fibres (sensory and / or motor) connect the lymph heart muscle fibres to the spinal cord above the penultimate vertebra. The number of fibres in the nerve labelled N1 is around 40. The sizes of the axons estimated from silver or osmium tetroxide stained cross sections of the nerve range from 0.3 μm to 4.0 μm in diameter. The diameter of the whole nerve N1 is approximately 200 μm. About one third of the fibres are contained within the smaller bundle of fibres within the nerve trunk. The other two thirds are contained in the larger bundle. Both of these subdivisions run parallel to form the single nerve N1.

FIGURE I.6a Light micrograph of end plates on lymph heart superficial muscle fibre. e., end plate. (Bromoindigo end plate stain, counterstained with Holmes-Blest reduced silver).

FIGURE I.6b Electron micrograph of neuromuscular junction between lymph heart superficial muscles and lymph heart nerve. Note the absence of extensive interdigitation at the myoneural junction and the abundant vessicles in the nerve. v., vessicles. (Prepared by and reproduced with the permission of Mr N.S. Greenhill).

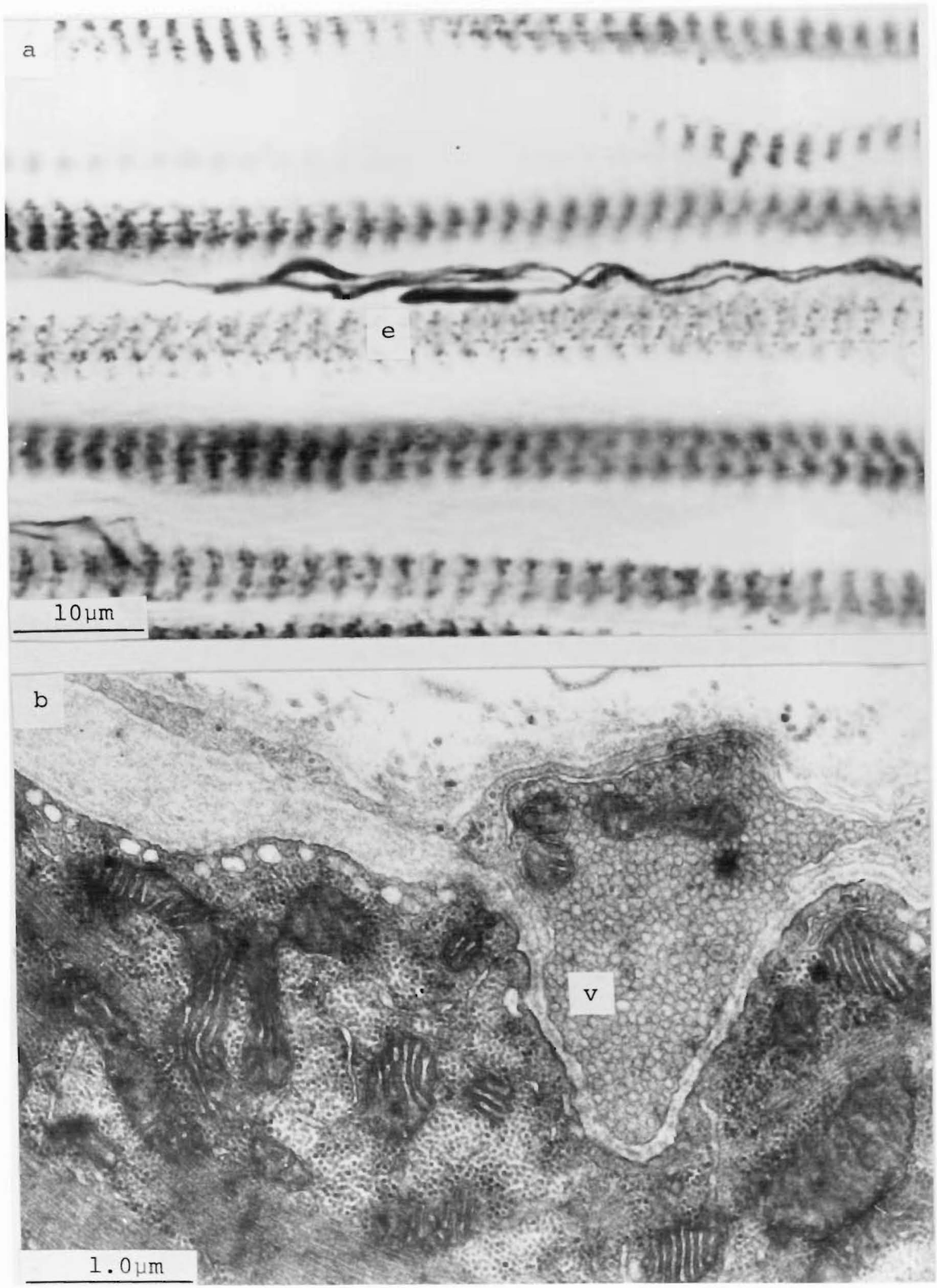
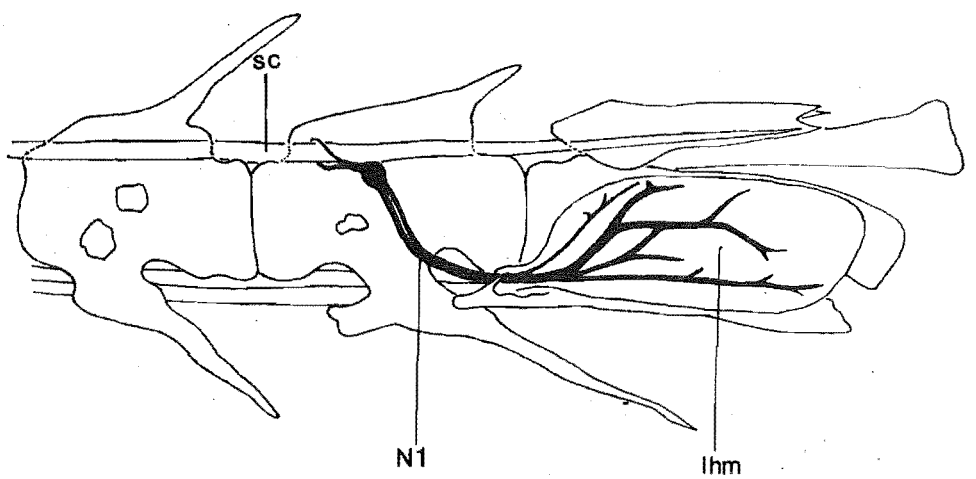

a
e
10µm
b
v
1.0µm

FIGURE I.7 Neuroanatomy of the short-finned eel caudal lymph heart. l.h.m., lymph heart muscle; Nl, lymph heart nerve; s.c., spinal cord.


SC
N1
lhm

I.5 Control of the Lymph Heart Beat

I.5.1 Introduction

As no previous attempt to study the control of the lymph heart beat had been successful (see section I.1.2) the problem was approached by three methods: ablation of the spinal cord and nerves near the lymph heart; application of selected pharmacological agents; and electrophysiological recording.

A simple isolated preparation was used for each of these studies. The preparation consisted of the last five to seven vertebral segments of the tail which were quickly removed from a freshly anaesthetised (0.04% benzocaine, H.F. Stevens & Co., Christchurch) or decapitated animal. This piece of tissue was placed into a dish of heparinized fresh water eel saline (see section II.4.2) where the skin was dissected free. The overlying muscle and connective tissue was removed and the tissue placed into a shallow constant temperature bath ($10^{o}C$) containing around 30 ml of fresh saline. Further dissection of tissues from the area to be examined was accomplished under a dissecting microscope. Benzocaine abolished lymph heart beat as did MS 222 (Chan, 1971). Contractions returned however after five to ten minutes in saline. Frequency of the lymph heart beat increases with temperature. Measurements of frequency of the isolated lymph heart and the lymph heart of resting intact live animals at a range of temperatures between $10^{o}C$ and $24^{o}C$ gave the following regression lines.

Isolated preparation $LHf = 8.96.T^{o}C - 69.3$

Intact animal $LHf = 8.20.T^{o}C - 57.2$

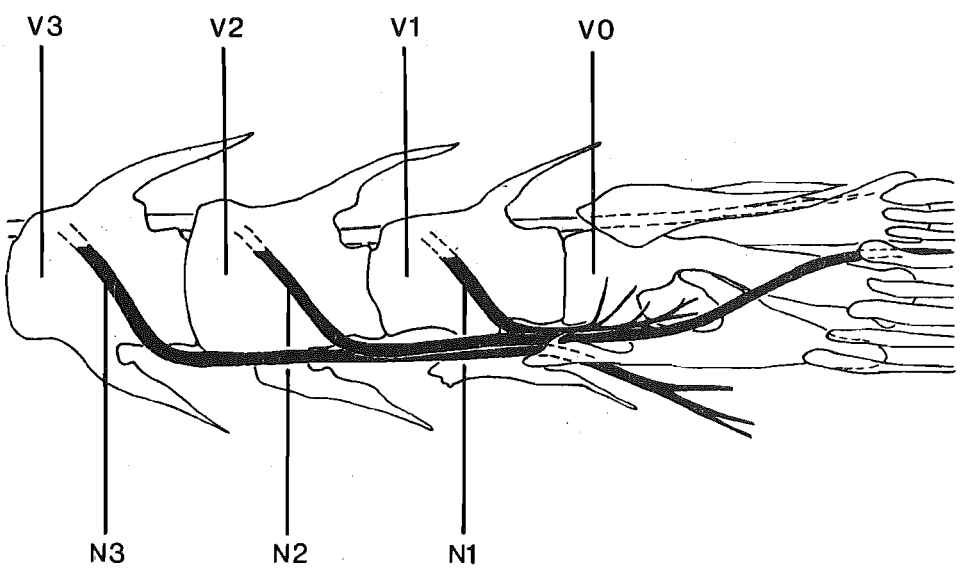
where LHf is lymph heart frequency and

T is temperature in degrees celsius, data from Davie (1975). Neither the slopes of the lines nor the intercepts of the two lines were significantly different. This indicates the suitability of the isolated preparation for the proposed experiments.

I.5.2 Ablation experiments

Once an isolated preparation was beating regularly, (~ 30 beats per minute at $10^{o}C$) nerves labelled N3, N2, N1 on one side (see figure I.8) were successively severed and the activity of the lymph heart observed. No change in activity could be observed until N1 was cut. When N1 was cut the lymph heart stopped, however a faint beat returned five to ten seconds after the ablation. This weak activity was passive movement of the muscles on the side being observed caused by contractions of the

FIGURE I.8 Diagram of the nerves of the last three vertebral segments of the eel tail. N1, lymph heart nerve, N2, nerve to caudal fin rays, N3, nerve to ventral fin rays, V0, the vertebra comprised of the hypural plates and the urostyle, V1, the penultimate vertebra from which N1 emerges; V2, the next cephalad vertebra from which N2 emerges; V3, the vertebra in the fourth from last segment from whence N3 emanates.


V3
V2
V1
V0
N3
N2
N1

muscle on the opposite side of the lymph heart. When only N1 was cut unilaterally, the same result ensued. Cutting N1 on both sides caused permanent inactivity. Clearly N1 is important in maintaining lymph heart activity.

Exposure of the cord was accomplished by carefully removing the connective tissue between the neural arches. Once removed the cord was transected between vertebrae V4 and V3, V3 and V2, V2 and V1, V1 and V0, (see figure I.14). No effect was observed when the cord was cut between V4 and V3, and V3 and V2. Cutting the cord between V2 and V1 always stopped the lymph heart beat. In two of the 14 preparations tested the beat returned after 10 to 15 minutes. Transection of the cord between V1 and V0 abolished the beat in all but two preparations, but contractions returned within 15 minutes in 12 out of the 17 preparations tested in this manner. The piece of cord from which N1 originates (dorsal and rostral to V1) is necessary for maintenance of lymph heart beat. Temporary abolition of activity which occasionally occurred when the cord was cut near V1, could be the result of traumatization of the cord in the area. A severe physical stimulus in the vicinity of the essential cells could easily disrupt normal functions.

I.5.3 Pharmacology of the lymph heart

Davie (1975) presented results from experiments involving topical application of certain pharmacologically active agents onto the isolated lymph heart preparation. A summary of these results is presented here.

Acetyl choline at 6×10^{-6} M induced small increases in frequency and amplitude. At higher concentrations, (6×10^{-5} M, 6×10^{-4} M) both amplitude and frequency were depressed.

Physostigmine at a concentration of 3×10^{-6} produced a brief increase in the force of contraction.

Serotonin, (5 - HT) had little or no action at 5×10^{-6} M or 5×10^{-5} M concentrations.

d-Tubocurarine at concentrations of 1×10^{-6} M and 1×10^{-5} M abolished contractions of the superficial muscle.

Decamethonium at 4×10^{-6} produced a transient contraction followed by quiescence.

Noradrenaline at 3 x 10^{-6} M, 3 x 10^{-5} M, and 3 x 10^{-4} M increased lymph heart amplitude but not frequency after a latency of ~ 40s.

These results, although from a preliminary investigation, suggest that the lymph heart receives excitatory cholinergic innervation, probably nicotinic, rather than muscarinic.

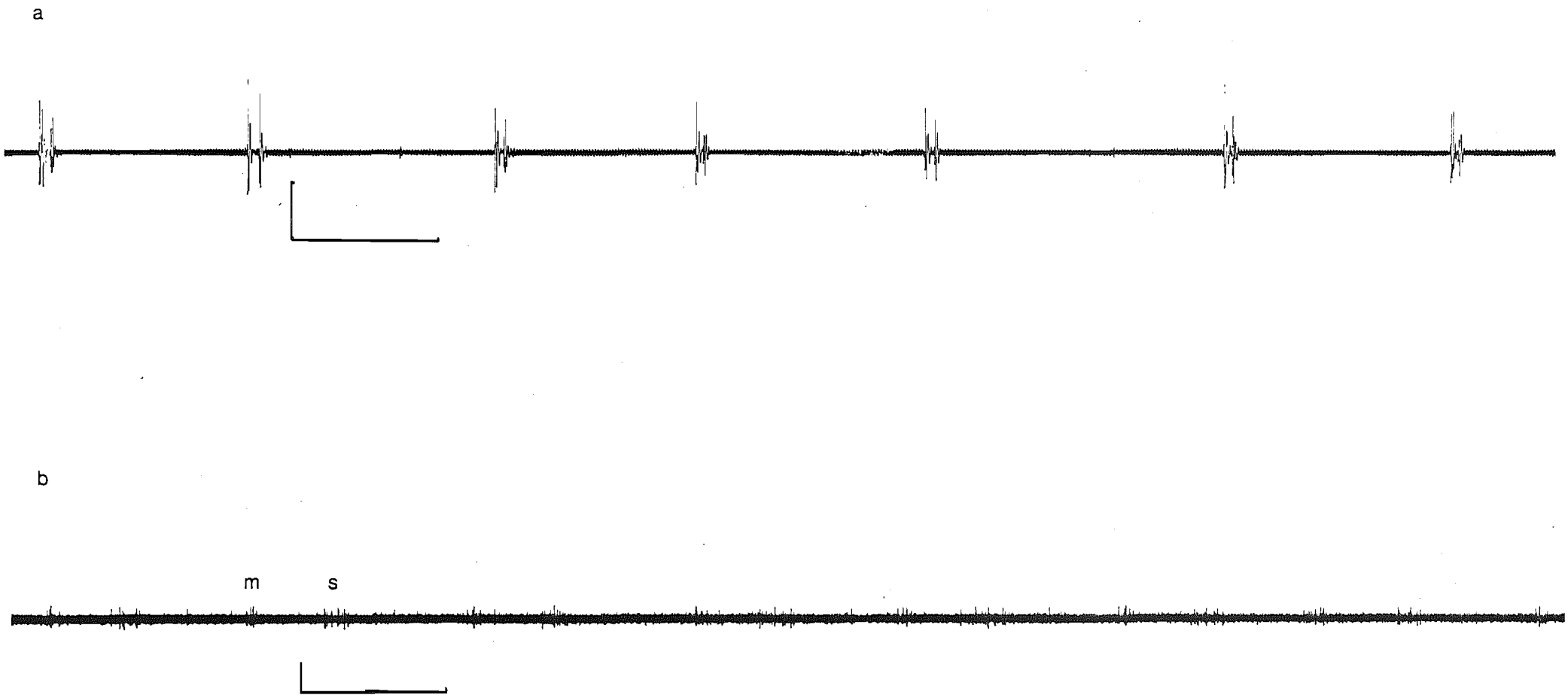
I.5.4 Electrophysiological recordings

The small size of the nerve Nl, (~ 200 µm in diameter), its inaccessibility and sensitivity to manipulations thwarted all but one of the 49 attempts to make 'en passant' recordings of neural activity. Unfortunately the muscle spikes were not recorded simultaneously. The successful recording apparatus was a tungsten hook electrode which could be insulated with mineral oil driven down a moveable sleeve by a remote syringe (Wilkins and Wolfe, 1974). Muscle records were taken with a suction electrode (see Delcomyn, 1974). Representative records of the muscle and nerve activity are presented in figures I.9a and I.9b respectively. Muscle activity was easily recorded with suction electrodes. In stable preparations two muscle spikes were regularly recorded, although occasionally three or four depolarizations were observed. The first spike or pair of spikes was larger than the second and coincided with superficial muscle contraction. The second muscle spike coincided with the contraction of the deep muscles. Both sides of the lymph heart contracted simultaneously. The time interval between the pair of spikes was 123.1 ± 17.9 ms (mean ± 1 SEM, n = 140. 10 cycles from 14 preparations which were beating at 33 to 37 beats per minute).

From the one set of satisfactory records from the nerve Nl it is clear that there are two sets of impulses per lymph heart beat. The group of 4 to 6, (4.58 ± 0.219, mean ± 1 SEM, n = 20) larger spikes occurred immediately before the onset of muscular contraction. The group of 6 to 10 (7.83 ± 0.405, mean ± 1 SEM, n = 20) smaller spikes which appeared in a less discrete group coincided with distension of the lymph heart between contractions. Two experiments were performed on this preparation. The nerve Nl was cut central to the point of recording and the larger (motor) spikes were no longer present in the record. However the rhythmic contractions of the muscle on the side opposite to that being recorded from caused regular distension of the passive muscles on the recording side. During each distension a regular, if somewhat different group of impulses were recorded from the

FIGURE I.9a Electrophysiological record of lymph heart muscle activity. Two muscle spikes were regularly observed, the larger first spike or spikes corresponded with contraction of the superficial muscle. The smaller second spike or spikes corresponded with contraction of the deep muscles. Vertical scale = 50 mV. Horizontal scale = 1 s. (Suction electrode).

FIGURE I.9b Electrophysiological recording of lymph heart nerve activity during lymph heart contraction. Two groups of action potentials occurred during a single cycle of lymph heart contraction. The smaller group labelled 'm' are designated motor spikes as they corresponded with contraction of the superficial and deep muscles. The larger group of smaller and less distinct spikes labelled 's' occurred during distension of the lymph heart between contractions. Although the records of muscle and nerve activity were taken from different preparations, at approximately the same temperature the frequencies are similar. The time scale of the two records are the same. Thus we can see that the motor spikes occur when muscle spikes appear on the muscle record but that the group of nerve spikes have no corresponding muscular contraction. Vertical scale = 10 mV. Horizontal scale = 1 s. (Oil insulated tungsten hook electrode).


a
b
m
s

nerve stump. Furthermore physical distension or stretching of the muscle blocks with forceps induced bursts of activity.

A detailed analysis of the results from a single preparation is not warranted, but these results show that the nerve N1 carries motor impulses to the lymph heart muscles from the spinal cord above the vertebra V1. The same nerve also appears to carry sensory information from the lymph heart to the spinal cord during distension.

I.6 Discussion

I.6.1 Discussion of results

The gross anatomy of the eel lymph heart has been accurately described and reviewed (Robin, 1880; Favaro, 1906; Kampmeier, 1969). The tissue layers and the positions of the muscle blocks are the same in the short-finned eel as in the european eel. However no reports of the fine structure of teleost lymph hearts or of their innervation are available.

Eel lymph heart muscle is striated and resembles fast acting skeletal muscle of many other vertebrates (Uehara, et al., 1976). The extensive transverse tubule system and the sarcoplasmic reticulum indicate rapid conduction of muscle depolarization throughout the muscle block. The sarcoplasmic reticulum is similar to that of the frog twitch fibres (Page, 1968) and it extends over the surface of the sarcomere length of the sarcomere and forms a fenestrated envelope. When compared to frog lymph heart muscle (Kawaguti, 1967; Schipp and Flindt, 1968) eel lymph heart muscle is more regularly arranged into muscle blocks. Frog lymph heart muscle is striated and is arranged in a three dimensional array within a connective tissue matrix. On contraction fibres must exert forces in a variety of directions.

The spinal cord above vertebrae V4 to V2 has typical teleost spinal cord organisation (Kappers, Huber, and Crosby, 1936). These authors stated that caudal dorsal root ganglia are located outside the neural canal in teleosts. No dorsal root ganglia were seen within the canal in sections from the eel but the group of cell bodies located outside the neural canal could be a sensory ganglion. Their general form however suggests that they are autonomic rather than sensory. The dorsal horn at the point of insertion of the dorsal root contains cell bodies that may be sensory. This point is still to be resolved.

The urophysis lies in the ventral cord above V2 and V1 and enlarges caudally to fill the whole cord above V0. The urophysis receives nervous input from higher central nervous centres which can initiate secretion of active principles (Fridberg and Bern, 1967). Chan (1975) found that urotensins I and II have hypertensive effects upon the japanese eel, and that urotensin II was the most potent. Urotensin II also caused increased frequency and amplitude of the caudal lymph heart. Hypervolemia caused release of urotensin II and or a lymph heart stimulating substance (Chan, 1971, 1975). Hypervolemia would of course cause excess lymph production, thus it is logical that lymph heart

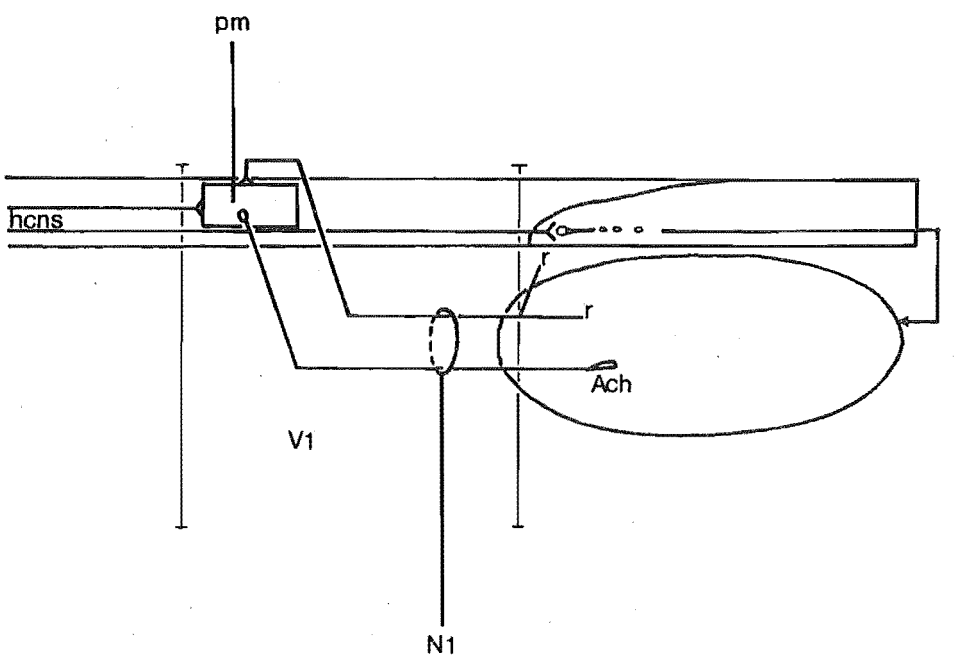
frequency and amplitude should increase to alleviate the load. Chan (1971) suggested that the urophysis is involved in the control of fluid balance in the tails of teleosts. Where caudal circulation is reduced as in seahorses (Syngnathidae), and ocean sunfishes (Molidae), there is little or no urotensin II activity, and lymph hearts are also absent. Recently Ichikawa (1978) tested teleost urophyses for acetylcholine and found very high concentrations. The reason for such an abundance of acetylcholine is not known. It is clear that the urophysis and the caudal lymph heart are involved in the return of fluid to the circulatory system from the tail.

Early experiments with teleost lymph hearts involved spinal cord destruction and suggested that they were myogenic (see Kampmeier, 1969). Mislin (1960) inferred indirectly that the eel lymph heart was under spinal control. The results from the present study show that spinal motor and sensory fibres traverse from the segment of spinal cord above the penultimate vertebra to the surface of the lymph heart muscle fibres. Some of these fibres, presumably motor, end on the muscle fibres in small 'en plaque' feet (see Couteaux, 1973; Uehara et al., 1976). These end plates can be visualized with a cholinesterase specific stain. The vessicles contained within the end plates are clear, spherical and of the same size as cholinergic vessicles. No vessicles of the smaller irregular granular type typical of aminergic terminals were seen in electron micrographs. The lack of interdigitation between the muscle and nerve membranes is atypical of fast skeletal myoneural junctions. Preliminary pharmacological evidence adds weight to the hypothesis that the excitatory neuromuscular transmitter is acetylcholine.

Electrophysiological recordings from the nerve N1 show two trains of impulses. One immediately preceeds the contraction of the muscles while the other is less discrete and composed of smaller spikes and occurs with distension of the muscle or filling. The latter persists after ablation of the nerve between the recording point and the spinal cord and probably emanates from some type of mechano-receptors perhaps in the tendons of the lymph heart muscles (Bone and Chubb, 1975). No stretch receptors were seen in any of the histological preparations.

I propose an hypothesis of lymph heart beat control as follows (see figure I.10). A pacemaker cell or neuropile located in the spinal cord above vertebra V1 initiates a train of impulses which are transmitted to the lymph heart via the nerve N1. The output from the pacemaker can be modified from feedback from stretch or other types of receptors in the lymph heart tendons. The nature of the receptors is

FIGURE I.10 Proposed 'wiring diagram' of the lymph heart of the short-finned eel. Motor action potentials are generated by a neural pacemaker (p.m.) located in the spinal cord in vertebra V1. These spikes are conducted via the lymph heart nerve N1 to the lymph heart muscles. Sensory information from receptors located somewhere in the region of the lymph heart (r) send information about distension to the spinal cord. Possible influence of higher central nervous system (h.c.n.s.) may act on the pacemaker or on the urophysis (u.). Liberated active principles from the urophysis (Urotensin II or lymph heart stimulating substance, (Chan, 1971)) may be delivered to the lymph heart via the blood or lymphatic systems.


pm
hcns
r
r
Ach
V1
N1

yet to be determined. Lymph heart frequency and amplitude can be modified by neural input from higher central nervous centres (see Berkowitz, 1956) either by acting directly on the pacemaker (Mislin, 1960) or by the release of urotensin II and/or lymph heart stimulating substance (Chan, 1971, 1975). Higher central nervous input is not essential to maintain lymph heart activity. The possibility of inhibitory innervation from the spinal cord as in the frog lymph heart also exists, (see Day, Rech and Robb, 1963).

I.6.2 Evolution of caudal vascular pumps

Fish locomotion by transmission of sinusoidal waves from the rostral to the caudal end of the body, increasing in amplitude toward the tail (Lighthill, 1971) appears to have presented problems to the circulation of body fluids within the tail. The effect of forces produced by these waves upon collapsable superficial vessels will be to force fluid to accumulate at the tip of the tail. Caudal lymph propulsors in fishes utilize swimming movements of the tail to propel lymph from the lymph propulsor into the caudal vein. This is accomplished by the water pressure that is created with each side stroke of the tail compressing the sinus, thus forcing the fluid through the valves. Anguilliform fishes derive considerable thrust from the dorsal and ventral caudal fins (Gray, 1933), which results in comparatively low hydrostatic pressure against the tip of the tail when swimming. This point was made by Allen (1910) and Kampmeier has taken the analysis one step further to suggest that the locomotory mechanism may have been the evolutionary cause of lymph heart development in anguilliform fishes. Various species have evolved different mechanisms to overcome the problem.

Within the elasmobranchs the Port Jackson shark (*Heterodontus portjacksoni*), has a series of valved veins which receive venous blood from the superficial vessels and direct it first deeper into the body, and then rostrally toward the caudal vein. Contractions of swimming muscles of the tail provide the propulsive force (Birch et al,, 1969). *Mustelus antarcticus* has a similar vascular arrangement except that the row of muscles that deflect the tail continue to contract in response to spinal motoneuron discharge during quiescence (Satchell, 1971).

In the Agnatha the caudal blood heart of the hagfish (*Polistotrema stouti*) has a pair of venous sinuses located either side of a median cartilaginous plate. Alternate contractions of the single muscle (musculari cordis cordalis, m.c.c.) on either side causes flexion of the

plate and subsequent expulsion of blood into the caudal vein. Greene (1899) stated that,

> "There is an automatic caudal heart centre located in the spinal cord which discharges rhythmic motor impulses to the caudal heart muscles."

The rhythmic motor impulses are transmitted to the m.c.c. by the third, fourth and fifth to last spinal nerves (Allen, 1917). Allen adds that up to eight muscle spindles are to be found in the m.c.c. which extend fibres along one of the nerve trunks to the spinal cord.

The situation is surprisingly similar for teleost lymphatics, rather than blood vessels. Many species have lymphatic sinuses which are emptied by the contraction of adjacent skeletal muscles. Fishes which have elongated body shapes commonly have active pumps to assist propulsion of lymphatic fluid into the blood stream. The short-finned eel has two lymphatic cavities located on either side of the tip of the tail which are encompassed on each side by three muscle blocks. These three muscle blocks form the lymph heart muscles which in every respect are similar to skeletal muscle and are probably derived from caudal tail muscles during devolution of the caudal fin. It is interesting to note that three spinal nerves from more vertebral segments innervate the caudal blood heart of the hagfish, but that only one muscle is found on each side. The m.c.c. is however differentiated into lateral and ventral portions (Greene, 1899). The lymph heart of the eel exhibits regular contractions and is not as sensitive to external stimuli, for example touch, as the hagfish caudal heart. No structures resembling sensory nerve endings were observed in any of the sections of the short-finned eel lymph heart. Sensory information does however emanate from the lymph heart. The source of these impulses is clearly not the teleost equivalent of the hagfish muscle spindles (Allen, 1917).

The fact that teleost caudal lymph hearts pump lymph suggests that with the development of higher blood pressures, blood recovery from the tail became less of a problem. The very low pressure lymphatic vessels, especially those that lie directly beneath the skin however would be vulnerable to the forces resulting from swimming. Thus the parallel evolution of strikingly similar structures in different fish taxa acting on different fluids under the same evolutionary pressures has resulted in the array of accessory caudal vascular pumps seen today.

I.7 Summary

1. The skeletal anatomy of the last three vertebral segments of the tail of the short-finned eel is described (section I.4.2).
2. Light and electron microscope studies of the three muscle blocks that comprise each side of the lymph heart show that the muscle is typical of vertebrate fast skeletal muscles (section I.4.3).
3. Muscles on each side of the lymph heart are innervated by a spinal nerve from the penultimate vertebral segment. This nerve contains approximately 40 axons which have diameters between 0.3 µm and 4.0 µm. Some of the axons merge with the spinal cord at the dorsal root and others at the ventral root indicating the presence of both sensory and motor fibres respectively (section I.4.4).
4. Neuromuscular junctions between the nerve from the penultimate vertebral segment and lymph heart muscle were visualized with a cholinesterase specific stain and are of the 'en plaque' type. At the electron microscope level the terminal nerve swellings contained spherical agranular vessicles of 40-60 nm diameter, suggesting that acetylcholine is the transmitter. Cholinesterase staining and preliminary pharmacology support the hypothesis that acetylcholine is the transmitter.
5. No stretch receptors were observed in any of the sections of the lymph heart muscle.
6. Electrophysiological recordings from the lymph heart muscle usually produced records of two muscle spikes. The first larger spike coincided with the contraction of the outermost layer of muscle while the second smaller spike coincided with contraction of the inner muscles (section I.5.4).
7. Only one set of electrophysiological records from the spinal nerve which innervates the lymph heart was obtained. This set of records revealed two trains of impulses in the nerve (section I.5.4). The group of four to six larger spikes corresponded with lymph heart contraction, whereas the group of six to ten smaller spikes corresponded with lymph heart distension.
8. Lymph heart contractions are under the direct control of spinal nerves emanating from the spinal cord located above the penultimate vertebra.
9. It is proposed that fish swimming movements restrict fluid return from the tail to the heart. The problem of vascular return from the tail of fishes is accentuated by elongated body form and low vascular

pressures. This problem appears to have been overcome by the development of a variety of accessory caudal vascular pumps in different groups of fishes. The caudal lymphatic heart of the eel is a specialised and well developed example of an accessory vascular pump.

PART II THE ISOLATED SALINE PERFUSED EEL TAIL

II.1 Introduction

Our understanding of the physiology of animals owes a lot to the study of isolated tissues. Much of our knowledge about cardiovascular functions and control has come from the study of isolated preparations. Two principal methods are applied to isolated tissue preparations; bathing or superfusion and perfusion. The first method is probably more fundamental. It involves suspension of thin strips or sheets of tissue from say heart or blood vessel in a bath of physiological salt solution. Responses to applied conditions (drugs, ions, osmolarity, temperature, etc.) are measured by the force or shortening produced during contraction or relaxation of the muscles. Such studies have provided the basis of pharmaco-dynamics in cardiovascular and other tissues where drugs have been applied. Some features of the cardiovascular system that are produced by smooth muscle activity are related to, but not directly calculable from this approach. Perfusion of tissues through their vascular network with a saline or blood allows the examination of many of the responses otherwise not accessible. Also larger pieces of tissue such as whole organs can be studied.

Responses to drugs are important in our understanding of control mechanisms within the cardiovascular system. When vascular tissue suspended in a bath is exposed to vasoactive agents, the rate of development of the response is often limited by the rate of diffusion of the agent through the tissue layers to its site of action. During perfusion we assume that transcapillary exchange is rapid (Landis and Pappenheimer, 1963), consequently the development of a response is limited by the delivery of the agent to the site of action; that is perfusion limited (see Waud, 1968). Despite the complexity of a perfused tissue compared to a bathed preparation it is intuitively a preferred method if the experimenter wishes to examine the effects of perfusate borne agents upon vascular parameters.

Resistance to flow is principally determined by the radius of the pre-capillary vessels (Mellander and Johansson, 1968). Radius changes are accomplished by changes in tension of the smooth muscle which surrounds these vessels. Although a bathed helical strip of artery will give values for tension changes in response to applied stimuli, direct measurements of resistance are not possible. Likewise vascular volume changes and capillary exchange processes can only be

studied if a perfused preparation is used. Resistance is defined as flow rate divided by the pressure differential across the vascular bed. From the Hagen-Poiselle equation for laminar flow of a newtonian fluid through a rigid tube of circular cross section,

$$Q = \frac{\Delta P \, \pi \, R^4}{8 \mu L}$$

Resistance is proportional to viscosity, μ, and path length, L, and is inversely proportional to the radius to the fourth power, R^4. Usually resistance is measured during steady state conditions where flow, Q, and pressure differential, ΔP, are recorded while other variables are held constant.

Two methods of perfusion are commonly used: Constant pressure with variable flow, and constant flow with variable pressure. A further method is to allow both pressure and flow to vary (Nichol, Girling, Jerrard, Claxton and Burton, 1951). Mellander and Johansson (1968) state that perfusion at constant pressure while flow varies, more closely approaches the '*in vivo*' state since in most animals blood pressure varies much less than does flow. These authors presented calculations showing that maximum resistance changes in skeletal muscle preparations during constant pressure perfusion were five times greater than maximum changes during constant flow. More recently Wood and Shelton (1975) compared maximum resistance changes with variable flow and variable pressure perfusion against maximum resistance changes with constant flow perfusion in the trunk of the rainbow trout (*Salmo gairdneri*). They found that maximum resistance changes were around four times greater for variable pressure and flow perfusion. Although maximum changes were greater for variable flow, variable pressure perfusion, Wood and Shelton (1975) found that pump perfusion (constant flow) resulted in higher resistance values at equivalent flows.

The behaviour of the cardiovascular system as observed in whole animals is the summation of all the circulatory events that occur in every tissue at that time. To study any one particular control system or response it is essential that as many as possible of the factors that could influence the measured parameter are held steady or measured accurately. For example, to measure the resistance change in an isolated perfused preparation following the application of a vasoactive drug it is essential that path length, viscosity and say flow are held constant while the pressure varies in response to the drug. Isolation of a vascular bed often results in the abolition of sympathetic nervous activity which can play an important role in

vascular tone maintenance (Su, 1975, 1978), thereby reducing one source of variability. Replacement of the blood with a Newtonian perfusate removes the effects of unknown concentrations of endogenous vasohumours and the anomalous viscosity properties of blood (Burton, 1965). In the absence of endogenous vasoactive agents, known amounts of substances such as catecholamines can be administered and their effects recorded. In the absence of motor nerve input, muscle activity in a skeletal muscle preparation will be reduced to a level approaching that of the basal metabolism thereby reducing the magnitude of the effects that metabolites will have on the local vasculature.

To extract useful information from a perfused vascular preparation all vascular parameters should be measured simultaneously. Where equipment or other limitations prevent monitoring of a wide array of events, as many as possible should be held constant. One feature of perfusion studies that emerges from the literature is that the objectives of the research should be clearly defined beforehand and the choice of tissue should be selective. Reasons for perfusing tissues in the past have included the study of transcapillary exchange processes, temperature regulation, gas and ion exchange and transport or simply to maintain the tissue to allow say neurophysiological recordings to be made.

Perfusion studies of teleost tissues have concentrated on the gills. Most gill preparations have utilized constant pressure perfusion (Keys, 1931: Rankin and Maetz, 1971; Payan and Matty, 1975; Haywood, Isaia and Maetz, 1977). Some branchial preparations have used constant flow perfusion (Keys and Bateman, 1932; Kirshner, 1969; Shuttleworth, 1972; Bergman, Olson and Fromm, 1974; Wood, 1975; Payan and Girard, 1977; Smith, 1977). By no means were all of these perfused gill preparations designed to investigate the vascular system of the gills since ionic and gaseous exchange processes are also located within the gills. The systemic vascular system of teleosts has been studied by isolated perfused techniques. Keys and Bateman (1932) perfused the whole body of the eel at constant flow as did Forster (1976a). The trout trunk preparation developed by Wood and Shelton (1975) excluded the branchial vascular bed and was perfused principally at constant flow. This particular preparation has yielded a great deal of very useful information (see Wood, 1976, 1977). Perfusion studies of the isolated swimbladder preparations have included those of Fange (1953) and Stray-Pedersen (1970). Both authors used constant pressure perfusion. An eel rete preparation perfused at constant flow was described by Stray-Pedersen and Steen (1975). Holmgren (1977) utilized a constant flow perfusion system in her isolated head-kidney heart preparation from the cod (*Gadus morhua*).

Isolated artery strips from fish have been studied by Kirby and Burnstock (1969), Burnstock (1969), Klaverkamp and Dyer (1974) and Holmgren and Nilsson (1974). Experiments with swimbladder strips have been performed by Fange, Holmgren and Nilsson (1976).

Constant pressure perfusion has seldom been used for fish systemic circulation studies. Although constant pressure perfusion may seem preferable on the grounds that it more closely approaches the natural situation, constant flow experiments have produced results that indicate that the perfusion conditions were most satisfactory and that the changes in resistance at constant flow were reliably measured (Wood and Shelton, 1975). Bergman et al. (1974) stated that perfusion of rainbow trout gills at constant flow by a peristaltic pump was qualitatively superior to perfusion at constant pressure (see Wood, 1975).

Vascular tone in teleosts has been considered to be controlled principally by circulating catecholamines (Stevens and Randall, 1967a; Randall and Stevens, 1967; Burnstock, 1969; Randall, 1970; Wood, 1974a; Wahlqvist and Nilsson, 1977), although this concept has been questioned more recently by Holmgren (1977, 1978) and Smith (1978). The presence of beta (dilatory) adrenergic receptors in the gills and alpha (constrictory) adrenergic receptors in the systemic vasculature of teleosts is well established (see Wood, 1974a; Wood and Shelton, 1975). Alpha receptors have been reported in the gills of some species, (Belaud, Payraud-Waitzenegger and Payraud, 1971; Bergman et al., 1974; Wood, 1974a). Whether or not beta receptors are generally present in teleost systemic vasculature is not clear. (Section II.6.1 summarises reports on the presence or absence of alpha and beta receptors in teleost systemic vasculature).

Responses of teleost systemic vessels to catecholamines have been intensively studied in one species, the rainbow trout, by Wood (1976). Holmgren and Nilsson (1974) however give a good account of the adrenergic system of the cod. The importance placed upon circulating catecholamines in the control of vascular functions in teleosts and the unresolved question of sympathetic nervous versus circulating catecholamine control indicates that more species should be studied. Despite the fact that teleost adrenergic receptors were first described from the european eel (*Anguilla anguilla*) by Keys and Bateman (1932) work comparable to that of Wood and Shelton (1975) and Wood (1976) on the systemic vasculature of the eel is lacking. There is however a large body of knowledge available about many aspects of the physiology of this commercially important family. When combined with the ready availability of eels

and their hardy nature they are attractive experimental subjects. Of the two species of eel available in New Zealand waters, the short-finned eel was arbitrarily chosen. The other species, the long-finned eel may well have been equally suitable (Shuttleworth, 1972).

The eel tail was chosen for perfusion for many reasons. The tail consists mainly of muscle as does the trunk of the trout. However a larger proportion of muscle is found in the tail and the visceral organs are excluded. In mammals the vascular reactions of visceral organs such as the liver, kidney and gastrointestinal tract to catecholamines are substantially different to the responses reported for skeletal muscle (see Mellander and Johansson, 1968). Thus a preparation including these organs may well be complicated by problems such as redistribution of blood and the production and catabolism of catecholamines by visceral tissues. In fish catecholamines are released into the venous system particularly the cardinal veins from interrenal tissue (Holmgren, 1977) and are thus ideally placed to affect the heart and gills. Fish veins are reported to be unresponsive to catecholamines (Burnstock, 1969). Endogenous catecholamines released into the veins of the perfused trout trunk preparation of Wood and Shelton (1975) are likely to have had little or no effect on the results.

Skeletal muscle appears to be an homogeneous tissue most suitable for perfusion as an organ. The presence of red and white or mosaic muscles (Hulbert and Moon, 1978) and their substantially different vasculatures (Gorkewicz, 1948) opens such a generalization to criticism. The post-anal tail of the short-finned eel represents approximately 45% of the animals total body weight and is almost entirely muscle. Because the tail represents such a large portion of the body, vascular adjustments within it are likely to have significant effects upon the whole circulatory system. In mammals the skeletal muscle beta vasodilatory response is a most important circulatory adjustment to exercise (Viveros, Garlick and Renkin, 1968; Rengo, Trimarco, Perez and Chiariello, 1976). During swimming teleost muscles build up lactate which is indicative of oxygen debt (Black, 1957; Driedzic and Kiceniuk, 1976) and suggests poor muscle blood supply. Webb (1971) maintained that in trout swimming power was provided by aerobic metabolism, consequently the need for adequate muscle blood supply indicates that the skeletal muscle vascular beds appear to be good subjects in which to examine systemic adrenergic receptors in teleosts.

The principal objectives of this section were to develop an isolated perfused eel tail preparation suitable for vascular research.

By the study of resistance responses to sympathetomimetic drugs the adrenergic receptors of the eel tail were to be classified. The adrenergic pharmacology of the preparation was to be compared to results from similar studies, especially those of Wood and Shelton (1975), Wood (1976) and Holmgren and Nilsson (1974). Pharmacological experiments were designed to yield information about vascular and non-vascular volume adjustments during resistance changes. To elucidate the function of the lymph heart, correlation of lymph heart activity with changes in non-vascular volume was planned to complement the results from the first section.

II.2 Caudal Circulatory Anatomy

II.2.1 Introduction

There are few published descriptions of eel tail vascular anatomy. Mott (1950a) described the gross anatomy of the arterial and venous vessels of the european eel. She described the course of the caudal vein to the kidney where it divides to form the renal portal vein 5-15 mm from the caudal limit of the kidney tissue. Variable numbers of anastomoses connect the renal portal and hapatic portal veins. Pollak (1957a,b) described in detail the arterial, venous and lymphatic vessels in the tail of the european eel. This study confirmed Dunajewski's (1930) description of the tail lymphatics of the same species but gave greater detail. Kampmeier (1969) reviewed the lymphatic system of vertebrates and provides a good account of the lymphatics of fish including the tail vessels of teleosts. Chan (1971) described the blood vessels in the area of the last three vertebrae of the asiatic eel (*Anguilla japonica*).

II.2.2 Methods

The caudal blood vessels of the short-finned eel were examined by dissection and by injection of a silicone rubber injection compound "Microfil', (Canton Bio-Medical Products, Boulder Colorado, MV112). Whole anaesthetised fish were perfused at 0.5 ml min^{-1} 100 g^{-1} through the ventral aorta with saline by a peristaltic pump, (Cole Palmer, Masterflex) until the effluent from the bulbus was blood free. The silicone rubber 'Microfil' was then prepared and pumped through the whole body. Alternatively the tail only was perfused via the caudal artery and filled with 'Microfil' in a similar manner. When the 'Microfil' emerged from the bulbus or the caudal vein the vessels or cannulae were clamped off and the tissue held at 4°C for 15 minutes. The spinal cord of whole animals was then cut just caudal of the opercula. Twelve to sixteen hours at 4°C was allowed for complete polymerisation of the 'Microfil'. The tissue was then cleared in a series of glycerine solutions of 20%, 50%, 90% for one to two days and then stored in 100% glycerine. Alternatively the tissue was etched in a 0.22% solution of pancreatin in 1.75% KOH for 48 hours at 37°C. The latter procedure was of little value since the skeletal components also disintegrated under the treatment and the cast collapsed.

The lymphatics were visualised by injection of 5% Patent Blue dye (α-Zurine, Sigma Chemical Co.) solution in saline subcutaneously (Gooneratne, 1974). Lymphatics selectively take up this dye

and appear bright blue.

One perfused tail preparation was perfused with a radiopaque material and X-ray photographs were taken during perfusion, (see section II.5.3). These plates gave information about the course of the larger arteries and veins in the tail.

II.2.3 Results

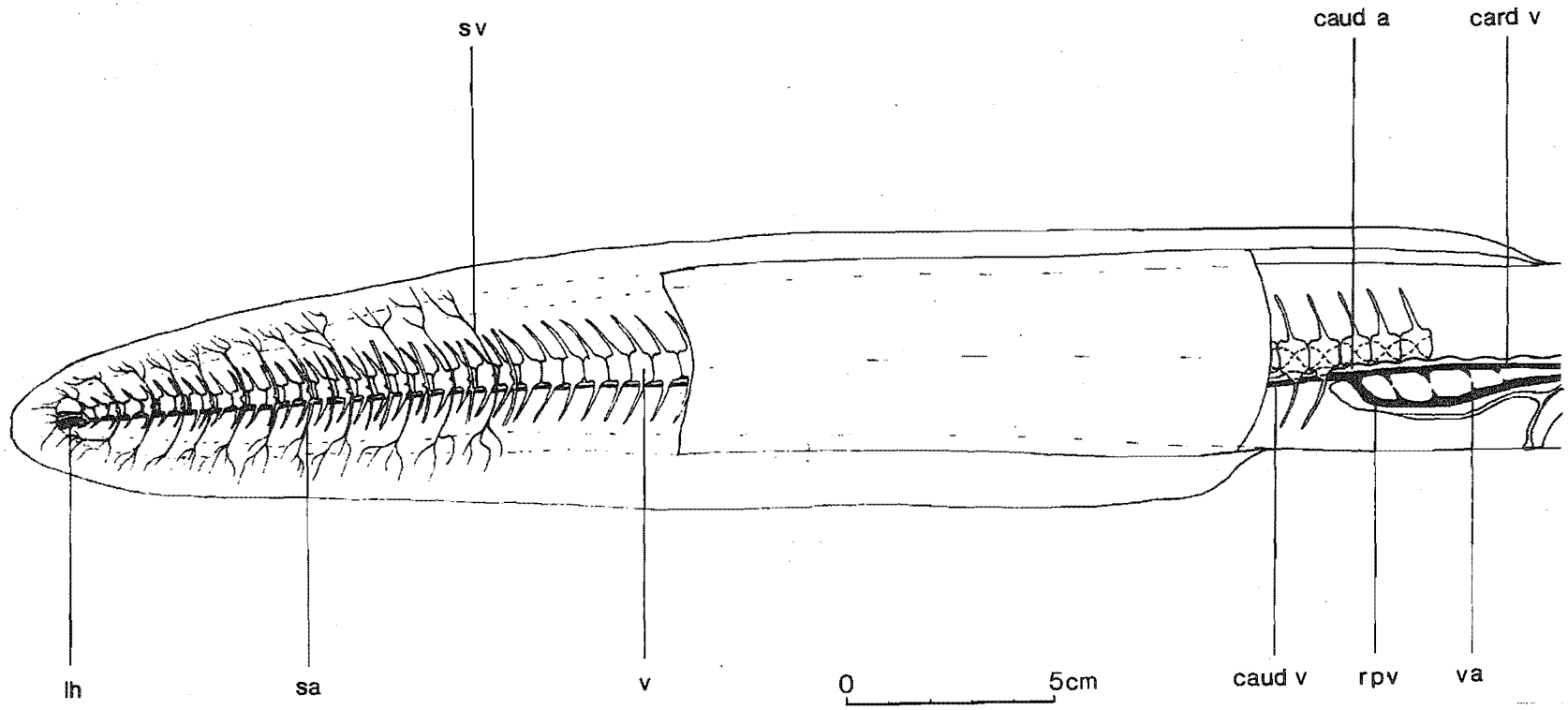
The positions and courses of the major vessels in the area just anterior of the haemal arch of the short-finned eel are essentially the same as described by Mott (1950a) in the european eel. The short-finned eel has a length of caudal artery and caudal vein just cephalad of the first haemal arch between four and ten mm long that is free of kidney tissue. This space is essential when preparing the tail for perfusion since at this point both vessels are ligatured once the cannulae are in place. In approximately one third of the tails prepared for perfusion there was a short venous anastomosis between the renal portal vein and the cardinal vein about five mm cephalad of the posterior limit of the kidney tissue. During preparation this vessel was cut between two fine ligatures tightened around it.

The five cleared 'Microfil' preparations and the X-ray photographs show that the segmental arteries and veins of the short-finned eel are arranged in a very similar manner to those of the european eel (see Pollak, 1957a). As with most fish vascular anatomy, individual variation in the size and number of vessels was present but to a lesser degree than experienced in the visceral region.

The blood vascular anatomy of the last three vertebral segments in this species is similar to the description of the area in the asiatic eel (*Anguilla japonica*), by Chan (1971) and european eel by Pollak (1957b). A diagram of arterial and venous systems of the short-finned eel tail is presented in figure II.1. Detail of blood and lymphatic vasculatures in the lymph heart area are presented in figure II.2. The muscle of the lymph heart is seen to have a very extensive blood supply compared to surrounding tissues.

The lymphatic vessels of the tail are extensive. The caudal lymphatic vessel lies ventral to the caudal artery and vein within the haemal arches. Glaser (1933, cited in Kampmeier, 1969) has shown that the caudal lymphatic is not a continuation of the cardinal lymphatic but is a separate vessel which connects cephalically with the circum-anal lymph plexus, and caudally with centrally directed branches from the two lateral lymphatics. Kampmeier (1969) stated that the caudal lymphatic

FIGURE II.1 Blood vascular anatomy of the short-finned eel tail. card.v., cardinal vein; caud.a., caudal artery; caud.v., caudal vein; l.h., lymph heart; r.p.v., renal portal vein; s.a., segmental arteries; s.v., segmental veins; v., vertebrae; v.a., venous anastomosis. Composite drawing from fresh specimens, 'microfil' injected specimens and X-ray photographs of radiopaque medium in the vasculature.


sv
caud a
card v
lh
sa
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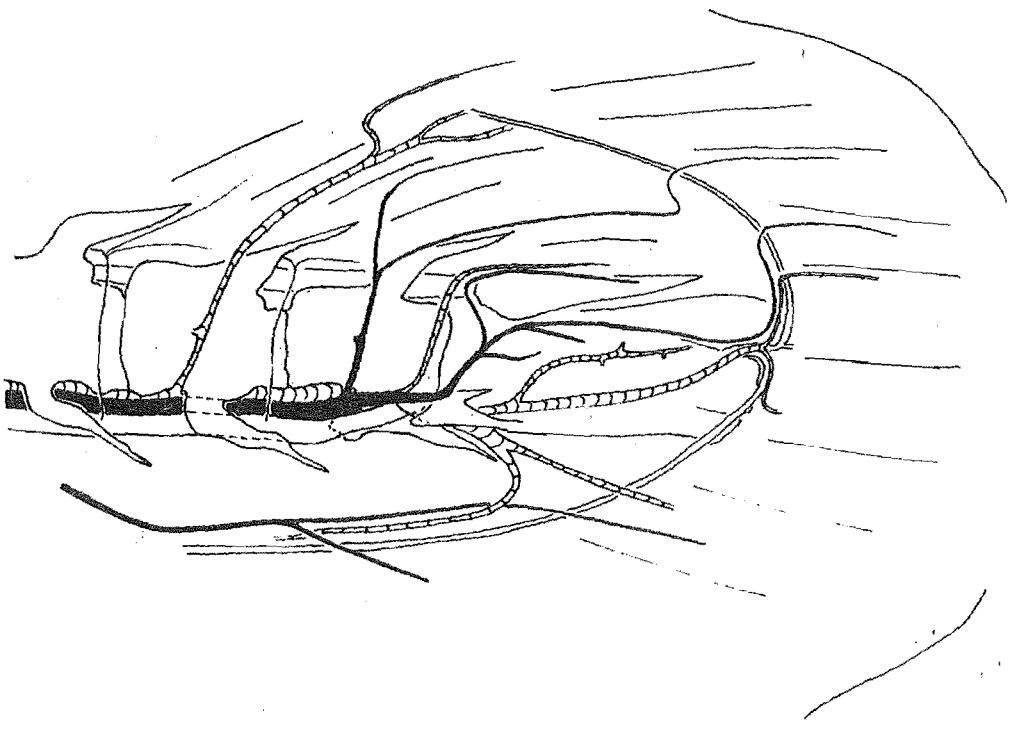
5cm
caud v
rpv
va

FIGURE II.2 Vascular anatomy in the area of the lymph heart. Arteries hatched, veins black and lymphatics unshaded.



1 mm

drains into the circum-anal plexus. Observations in the short-finned eel indicate that this vessel empties at least part of its contents into the right hand side of the lymph heart as described by Favaro (1905). At the junction of the caudal lymphatic with the lymph heart, the vessel is about 1.5 times the diameter of the caudal vein, (see figure II.2). The caudal fin lymphatic connects the caudal fin lymph sinus and the lymph heart. This vessel passes through the narrow gap between the thickened posterior extensions of the hypural plates. The caudal fin lymph sinus collects lymph from the fin ray lymph vessels and from the lateral lymphatics. The caudal lymphatic vessel and the lateral lymphatics both have more than one lymphatico-venous connection.

Injection of indian ink into the left side of the lymph heart fills the caudal vein but not the right side of the lymph heart. Likewise, injection into the right side of the lymph heart fails to fill the lymph vessels that empty into it. Valves between the two chambers of the lymph heart and at the points of entry of the caudal and caudal fin lymphatics have been observed in histological preparations of the area. A further valve at the lymphatico-venous confluence of the lymph heart and caudal vein was found to be larger and thicker than the other three valves. All the valves appeared to be bicuspid.

II.3 Perfusion of the Eel Tail. Methodology

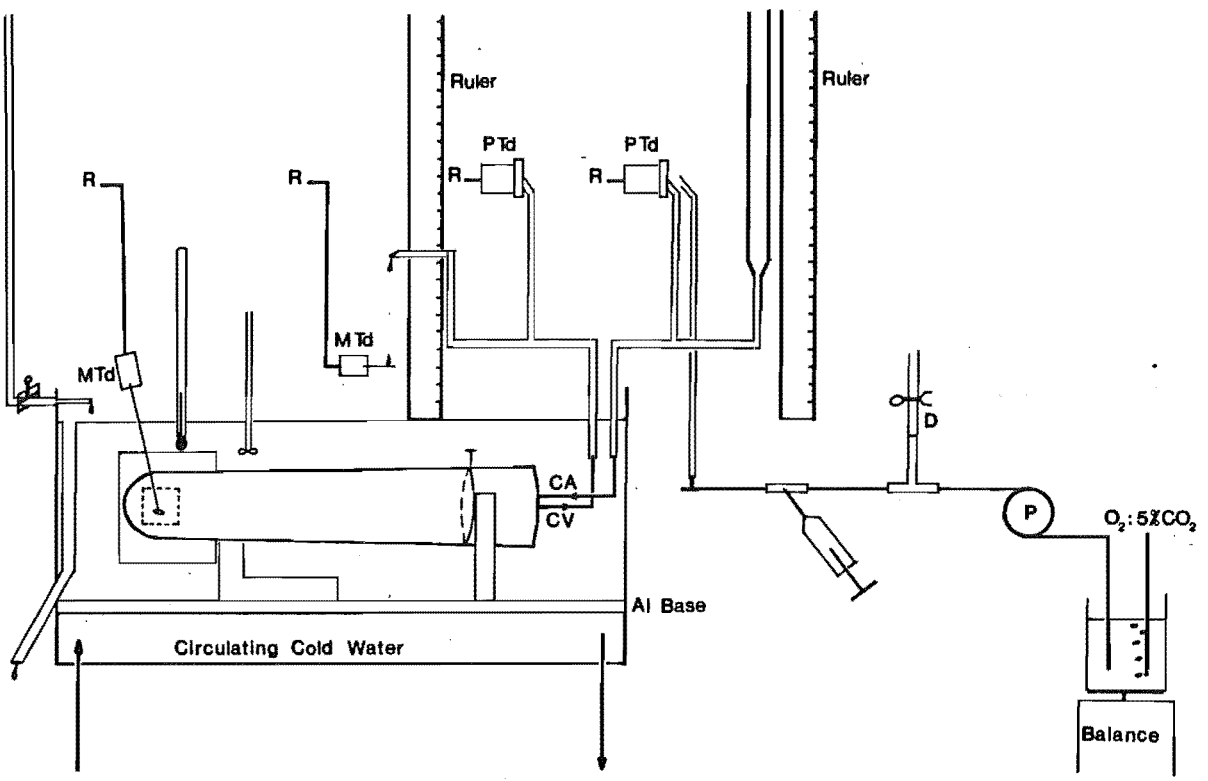
II.3.1 Preparation of the eel tail for perfusion

Short-finned eels between 270 and 719 g (461.8 ± 5.7 g; mean ± 1 S.E.M.; n = 86) were anaesthetised in 0.04% aqueous benzocaine, (Wedemeyer, 1970). Anaesthesia took 9.9 ± 0.4 minutes (mean ± 1 S.E.M.; n = 86). The fish were laid ventral side up on a dissection bench at room temperature and the bulbus arteriosus exposed. Heparin (ammonium salt, Sigma Chemical Co.) was administered at 500 iu per 100 g body weight in 1.0 ml of saline and allowed to circulate for five minutes. Blood was then withdrawn from the bulbus and stored in the syringe at 4°C if required later. A ventral incision about 30 mm long was made in the body wall to the left of the midline; ~ 20 mm caudal of the vent. The kidney tissue was carefully dissected from the connective attaching it to the body wall and the caudal artery and veins exposed to their point of entry into the haemal arch. The front of the eel was then cut from the tail just caudal of the vent. Two cotton ligatures and one silk thread were placed around the caudal artery before cannulation. One cotton thread was placed around the caudal vein before cannulation. Portex ppl60 cannula (120 mm long) was used to cannulate both vessels. Cannulae were prefilled with heparinized saline, (100 iu per ml). The cannulae were inserted until they were ~ 20 mm caudal of the first haemal arch, the arterial cannula a millimetre or so more caudal than the venous cannula. The cannulae were tied in and a further silk ligature was tied about the caudal vein and the kidney to which it is attached.

A tourniquet of size 2 silk was placed about the body just caudal of the first haemal arch and was tied tight. The preparation was connected to a peristaltic pump (Cole Palmer Masterflex, Cole Palmer 6408 - 41 tubing) and exsanguinated (5-10 minutes), with heparinised saline. The tail was then weighed and placed in the 7 ℓ constant temperature saline bath, (10 ± 1°C). Once in the bath the tail was sutured to a yoke which supported the cephalad end (see figure II.3). The tip of the tail was pinned over a wax block which had the centre cut out. The hole in the posterior support allowed a light to be shone through the tissue so that skeletal elements in the tail could be recognised. The lymph heart recorder could then be accurately micromanipulated into place.

Preparation time from the end of anaesthesia until the preparation was exsanguinated was 31.3 ± 0.4 minutes (mean ± 1 S.E.M.; n = 86). No experiment was continued for more than six hours. After the end of

FIGURE II.3 Diagram of experimental apparatus used for perfusion of the eel tail. CA, caudal artery cannula; CV, caudal vein cannula; D, depulsator; MTd, mechanotransducer; PTd, pressure transducer; P, peristaltic pump; R, to recorder; T, tourniquet.


Ruler
Ruler
PTd
PTd
R
R
R
MTd
MTd
CA
CV
D
P
O_2: 5%CO_2
Al Base
Circulating Cold Water
Balance

each experiment the tail was reweighed and all equipment was soaked for 12 hours in 'Pyroneg' (Diversery Wallace Ltd., Papatoetoe, Auckland) before washing, rinsing in distilled water and drying at 80°C.

II.3.2 Perfusion methods

Two perfusion methods were used. The first consisted of a vertical tube of known volume per unit height (1.11 ml per 10 mm) which was filled with saline. When connected to the preparation via the arterial cannula the fluid was allowed to pass through the vascular bed and out the venous cannula. As the level of saline in the tube fell, the input pressure fell. Change in pressure is directly related to flow. Hence from the record of pressure with time, flow was calculated.

The second perfusion method was by means of the aforementioned peristaltic pump. Flow rates were calculated from the change in weight of the reservoir as measured by a Mettler top loading balance, (p 1200). The flow rate was set at 0.5 g of saline per 100 g of tail tissue weight. Mean measured flow rate was 0.495 ± 0.002 ml per 100 g tail tissue (mean ± 1 S.E.M.; n = 86).

The saline delivery system contained a bubble trap constructed from a nylon T piece of low volume (~ 0.5 ml), which by regulation of the gas space at the top allowed adjustment of the 'pulse' pressure. The traditional inverted vial used as a bubble trap was unsatisfactory because mixing of the incoming saline with that in the vial was incomplete. When perfused with an Evan's blue dye solution, the system used delivered from the venous cannula a dye concentration of 95% of the input solution within 150 s. In just over 30 s, 50% of the dye solution concentration was produced. After four minutes the concentration stabilized at 98% to 100% of the initial concentration. The inverted vial only delivered 85% of the reservoir concentration after 12 minutes. These data are important when considering the time course of the responses of the preparation to drugs delivered by this system.

A stainless steel injection port of low volume (~ 0.05 ml), was constructed from a 'Gordh' 20 gauge intravenous needle fitting, (Eschmann, England). This device allowed replacement of the seal, (No 789168), after each preparation and had a very small dead space. A coil of tubing in the bath allowed equilibration of bath and perfusate temperatures.

II.3.3 Measurement of perfusion parameters

Flow. During vertical tube perfusion flow was calculated by drawing a tangent to the pressure record with time at the desired pressure and calculating flow from the linear relation between pressure and flow in such a set up. Flow is thus the dependent variable.

For pump perfusion the change in reservoir weight with time, when corrected for specific gravity of the perfusate (1.0090 $g.ml^{-1}$) gave the flow. Because the reservoirwas constantly bubbled with 95 O_2/5% CO_2 evaporation may not be insignificant.

Outflow in drops was recorded by placing a ceramic gramophone cartridge (BSR X5H, Phillipps), in the path of the falling venous outflow. Each time the drop deflected the stylus, the piezioelectric crystal produced a signal which was displayed on the recorder. Drop weight varied between preparations because of different absolute flow rates and was measured gravimetrically for each preparation.

Pressure. Both input and output pressures were measured with Bell and Howell type 4-327-0010 pressure transducers which were filled with saline and calibrated against the internal standard in the recorder. Checks on this calibration were made periodically with a column of saline connected to the other port of the transducer head. The level of the saline in the bath was maintained by a constant inflow and overflow and was taken as zero pressure.

Lymph heart. During perfusion it was not possible to see movements of the lymph heart as in the intact animal because of the absence of blood. To record lymph heart beat a fine glass extension was fitted over the end of a Devices 4751 100 g force displacement transducer. When the glass extension was lightly rested against the area of skin lateral to the lymph heart the body wall movements produced a record. This mode of recording did not allow accurate or absolute measurements of amplitude. Consequently any changes in amplitude are relative to those before experimental manipulation. Frequency of the lymph heart was reliably and accurately recorded.

Oxygen tension. Oxygen tension in the saline was measured with a Beckman Field Lab Oxygen Analyser with 3995 sensor. Reservoir and 'venous' oxygen tensions were measured in 12 preparations.

pH. pH of the perfusion medium was determined with a Metrohm E488 pH meter.

All parameters except pH were recorded continuously on a Devices

MX4 four channel recorder for full analysis after the experiments.

II.3.4 Drugs and chemicals

Drugs used in experiments on these preparations were: Heparin, ammonium salt (Sigma Chemical Co.) l-adrenaline, free base, (Sigma Chemical Co.), l-noradrenaline, free base (Sigma Chemical Co.), l-noradrenaline, bitartarate (Koch-Light Laboratories), l-isoprenaline, bitartarate (Sigma Chemical Co.), phentolamine, mesylate (CIBA), propranolol, (ICI Ltd., Pharmaceuticals Division), dichloroisoproterenol, hydrochloride (Eli Lilly and Co. Ltd., Bassingstoke, England).

II.4 Ringer

II.4.1 Saline

The saline solution used throughout this study was fresh water eel Ringer's solution after Rankin and Maetz (1971). Glucose and polyvinylpyrrolidone were omitted from the bathing ringer. All salines used for perfusion were vacuum filtered through Watman GFC filter paper immediately before use.

Eel plasma was collected from five eels and Na^+ and Cl^- concentrations determined. Sodium concentration was measured with an Eel flame photometer and chloride concentration was measured with a Corning Eel Chloride meter, (potentiometric titration). Mean Na^+ concentration was 0.142 ± 0.001 M (mean ± 1 S.E.M.), which is close to the Na^+ concentration in the ringer of 0.158 M. Mean Cl^- concentration was 0.111 ± 0.001 M (mean ± 1 S.E.M.) compared to 0.119 M in the saline.

II.4.2 Viscosity

The viscosity of the saline was increased with polyvinylpyrrolidone (PVP) of 360,000 average molecular weight (Sigma Chemical Co.), to a value near half way between the whole blood and plasma viscosities (see Whittaker and Winton, 1933; Wood, 1974a). Blood was collected in dried heparinised syringes from the bulbi of nine eels and was pooled in the case of three pairs thus obtaining six samples. The mean haematocrit of the six samples was 40.15 ± 0.93%, (mean ± 1 S.E.M.) which is slightly higher than reported by McArthur (1977) for this species. To measure the viscosity the blood samples were passed through an Oswald viscometer with a bore diameter of 0.428 mm. This size bore was calculated to have less than 1% effect on the relative viscosity of the solutions (Burton, 1965). The mean time taken for the blood to pass through the viscometer was 1138 ± 69.7 s (mean ± 1 S.E.M.) giving a relative viscosity of 6.05. The blood was then centrifuged at 5000 rpm for ten minutes and the plasma taken off. Two pairs of plasma samples were pooled leaving four samples. The mean time taken for plasma to pass through the viscometer was 407.0 ± 21.4 s (mean ± 1 S.E.M.; n = 4), a relative viscosity of 2.15. The viscosity of distilled water and solutions of 8.6 gl^{-1}, 10 gl^{-1}, 15 gl^{-1} and 20 gl^{-1} PVP were measured. Half the difference between the time taken for whole blood and plasma is added to the time taken for plasma which gives a time of 772.5 s, or a relative viscosity of 4.10. This is very close to the time for the 10 gl^{-1} PVP solution. Subsequently 10 gl^{-1} PVP was used to increase

the viscosity of the ringer to near physiological values.

Specific gravity of short-finned eel blood and ringer with 10 gl^{-1} PVP added were calculated to be 1.0503 gml^{-1} and 1.0090 gml^{-1} respectively.

Human serum was supplied by the Haematology Laboratory Christchurch Public Hospital. Addition of 5% serum to the saline did not significantly alter its viscosity.

II.4.3 Experimental protocol

For each preparation no drugs were introduced until after the measurement of flow/pressure differential profiles, baseline resistance and changes in resistance with venous pressure. These measurements were only made after the preparation had been perfused for 15 to 30 minutes to allow a period of stabilization. Before each set of drugs and intermittently between various concentrations blank saline samples were prepared and administered in the same manner as drugs. Administration of blanks and drugs was by the perfusion line (infusion) or as a bolus of 0.1 ml (injection). None of the blanks produced significant responses. Dose response curves were calculated from the responses to cumulative addition of increasing concentrations (van Rossum, 1963). In no case was a dose response curve constructed from a preparation treated with any other drug. For the experiments involving antagonists about half the preparations had previously had dose response curves constructed from their responses.

II.4.4 The use of human serum in the saline

Regulation of blood flow through vascular beds requires that the resistance to flow can be increased or decreased. Isolated saline perfused vessels and some vascular beds often assume a baseline resistance that is equivalent to maximal dilation, that is complete relaxation in the absence of any vascular tone (Waugh and Shanks, 1960; Bohr and Johansson, 1966). From this baseline it is impossible to elicit any further dilation. Therefore the *in vivo* situation must surely be a normal tone near the midpoint resistance to achieve effective regulation. Vascular tone is defined by Mellander and Johansson (1968) as "... the average level of contractile state in the musculature within a region or section of the peripheral vasculature." Folkow (1960) stated that circulating vasoconstrictor substances play an insignificant part in the maintenance of tone in mammals. He maintained that the

sympathetic nervous system played a dominant role in tone maintenance. There are however many reports of non-neurogenic tone in isolated perfused vascular preparations which is the result of unknown vasoactive substances in the blood (Bohr, Verrier and Sobieski, 1971).

Isolated mammalian perfused kidney, skeletal muscle, and blood vessel preparations have been the subjects of intensive study of autoregulation of vascular resistance (Waugh and Shanks, 1960; Stainsby, 1964; Johnson, 1964). One important finding of these investigations was that effective regulation of blood flow was maintained by the presence in the perfusate of small amounts of plasma or serum. Cardioactive substances from plasma of between 4000 and 8000 mwt have been described by Naylor, Robertson, Price and Lowe (1965). Bohr et al. (1971) reported an even lower molecular weight fraction from protein-free plasma that maintained skeletal muscle resistance vessel autoregulation. Very large proteins, cardioactive globulins, were described by Hadju and Leonard (1961). Bullivant (1978a,b) described the use of human serum and bovine serum albumin to maintain autoregulation in isolated perfused rat kidneys. It appears that the addition of plasma to the perfusate confers upon isolated perfused preparations characteristics that are observed in whole intact animals. Extensive observations by Waugh and Shanks (1960), Hadju and Leonard (1961), Stainsby (1964) and Bohr et al. (1971) have shown that the multiple effects of plasma on perfused preparations are not the result of the cellular fraction but are elicited by plasma, serum and protein free serum. The effects of these various fractions of plasma are not similar to the effects of any vasoactive agents so far described (Bullivant, pers. comm.). Catecholamine blocking agents and metabolic inhibitors had little or no effect on the serum induced tone (see Bohr et al., 1971).

Plasma in the perfusate at low concentrations (0.25 to 1.0%) also had the ability to potentiate the responses to ions (K^+, Ca^{++}) and humoral vasoactive agents (noradrenaline, angiotensin), (Bohr and Johansson, 1966). Recently Smith and Rozengurt (1978) have discussed the molecular basis for the effects of serum proteins upon membrane function. They maintain that certain ionophores present in serum increase membrane permeability to Na^+. In muscle cells, increased intercellular Na^+ would lower the threshold for contraction. Thus more muscle cells are likely to be recruited for a given stimulus, enhancing the response.

Blood proteins, particularly albumin, have been suggested to have transport or protective functions for labile vasohumours. Callingham

and Barrand (1976) stated that catecholamines are more stable in plasma than in saline. Binding of catecholamines to albumin is slow (> 18 hrs) but a significant proportion (> 40%) occurred immediately. The half life of noradrenaline and adrenaline in mammalian blood has been estimated to be around 20 s (Ferriera and Vane, 1967). The blood bath technique and bioassays used by Ferriera and Vane mean that they measured the amount of active drug available to the tissue. That greater quantities are present but are unassayable, perhaps because of protein binding, remains a possibility. Binding of drugs to plasma proteins and tissues increases the half life of other drugs (Gibaldi, Levy and McNamara, 1978) and increases the apparent volumes of distribution (Gibaldi and McNamara, 1978). A chemical equilibrium between plasma protein-drug complexes and free drugs which are accepted to be the active agents, and receptor-drug complexes is conceivable which could explain the prolonged action of drugs despite the rapid disappearance of the assayable free fraction. Busacker and Chavin (1977) studied the uptake and turnover of radio-labelled catecholamines in goldfish. They were quick to point out that they followed the radiolabel of the drugs which may not reflect the movements of the active agents. They stated that degradation products of adrenaline and noradrenaline are not known to be taken up by the tissues examined. Subcutaneous injections of low doses of adrenaline and noradrenaline resulted in rapid (< 2 min) appearance of the catecholamines in the plasma. The interesting point is that both adrenaline and noradrenaline persisted in the plasma for long times. Plasma adrenaline levels were maintained for 128 minutes and plasma noradrenaline levels peaked at 32 minutes after injection.

Fish have very permeable capillaries, (Hargens, Millard and Johanssen, 1974). An increase in capillary blood pressure would result in extravasation of fluid and subsequent oedema. Thus we could expect either a strict control of blood pressure or an efficient means of tissue fluid recovery. Changes in colloid osmotic pressure represent less of a problem in fish. Leaky capillaries would result in rapid equilibration of blood and tissue colloid osmotic pressures. This would relieve the need to alter blood pressure in response to blood protein concentration changes. Myhre and Steen, (1977) reported that albumin and horse serum assist in maintaining normal capillary permeability in the perfused eel rete preparation. Landis and Pappenheimer (1963) suggested that serum proteins bound loosely to capillary endothelium. Levick and Michel (1973) supported this idea and Fry, Mahley, Weisgraber and Oh (1977) have measured the amount of labelled albumin bound to canine aortic walls.

The binding of albumin appears to be non-uniform, localised at the cell junctions, the purported sites for pores (Landis and Pappenheimer, 1977). Hadju and Leonard (1961) found that their globulin fraction could not be washed out from a heart that had been exposed to them. Movement of proteins through capillary walls, the most significant barrier to their movement (Courtice, 1971), is generally accepted to be through pores. Many models of pores including their sizes and areas in capillaries have been suggested (see Landis and Pappenheimer, 1963; Stray-Pedersen and Steen, 1975). The endothelium cell junction appears to be the most likely site for such pores. The model proposed by Stray-Pedersen and Steen (1975) has many attractions in that the large pore filled with fibrous material can act as a molecular sieve. This model offers explanations as to the mode of action of the plasma proteins in maintaining permeability. It is easy to conceive of a matrix of macromolecules being altered to allow the passage of various sized molecules to pass, and for plasma proteins to be part of such a matrix or 'filtercake', (see Mason, Curry and Michel, 1977).

This information about the actions of blood proteins upon the vascular system indicates that an investigation of an isolated perfused vascular preparation that is concerned with resistance, the responses to catecholamines and the production of extravascular fluid could benefit from the addition of serum or blood proteins to the perfusate. The isolated perfused eel tail is just such a preparation and 5% human serum has been added to the perfusate in 36 of the preparations.

II.5 Results

II.5.1 General results

One hundred and one isolated perfused eel tail preparations were prepared. Twelve preparations were discarded because venous outflow was less than 80% of the inflow (efficiency of perfusion). Three were discarded because gas bubbles were inadvertently let into the perfusion line. The results presented here are from 86 satisfactory preparations.

Units used in the results follow the suggestions of Kappagoda and Lindin (1976) for the use of SI units in cardiovascular research. Consequently all pressures (P) are in kilopascals (kPa), flows (Q) in $ml.min^{-1}$, resistances (K) in $kPa.ml^{-1}.min$, and drug concentrations in molar (M) concentrations or doses. The free base was taken for the molecular weight of the drugs. Pressure differential (Pd) was measured across the vascular bed from the caudal artery to the caudal vein. Changes in any of the above parameters are indicated by delta (Δ). All values are quoted as means plus or minus the sample standard error of the mean, unless otherwise stated.

The tissue perfused amounted to 45.15 ± 1.53% (n = 86) of the total body weight of the fish. This calculation includes the small amount of tissue at the anterior end of the preparation which was excluded from perfusion by the tourniquet. The efficiency of perfusion was 87.16 ± 2.31%, (n = 86). In twelve of these, at the end of experiments, the input and output cannulae were connected by a short length of tubing and inflow and outflow recorded. The mean value was 96.35 ± 2.61%. This somewhat lower than expected value for maximum efficiency indicates that not all the fluid that is lost from the reservoir emerges from the venous cannula. As noted before evaporation may be significant.

All tails gained weight during perfusion, the mean increase was +12.48 ± 1.85% (n = 86). Perfusion stimulated mucus production and upon wiping the excess slime off 11 preparations the weight gain was reduced to +7.59 ± 1.45% (n = 11). Although there was an increase in tissue weight there was no concommitant increase in baseline resistance with time.

After all experiments were completed the input and output cannulae were dissected free with the ligatures still in place and connected by a short length of tubing (short circuit perfusion). The pump setting was not altered and the resistance of the delivery and collection system was measured. The mean system resistance (Ks) was 0.619 ± 0.85 $kPa\ ml^{-1}$ min (n = 86) and was equivalent to about 40% of the mean baseline tail

vascular resistance. This quantity was measured for each preparation and was subtracted from each resistance measurement (see Wood, 1974a).

Deterioration of perfused preparations as indicated by increasing baseline resistance with time of perfusion has been a problem in previous investigations (see Rankin and Maetz, 1971; Wood, 1974a). In the perfused eel tail no such problems were encountered. Filtration of the medium through Whatman GFC glass paper was adequate to remove the problem of slowly increasing baseline resistance caused by particulate matter. In several preparations where no experiments were performed for several hours, a very slight decline in baseline resistance (< 2%) was observed. Baseline resistance was taken between 30 minutes and one hour after the start of perfusion when a stable level was achieved.

II.5.2 Baseline resistance

Pump perfusion of 50 tails with the basic saline, (fresh water eel saline plus 10 $g\ell^{-1}$ PVP), was performed at venous pressures of zero and 1.33 kPa. Thirty six preparations were perfused with 5% human serum added to the perfusate at a venous pressure of 1.33 kPa. These experiments gave the mean baseline resistances tabulated below (see table II.1).

Table II.1 Baseline resistance of isolated saline perfused eel tails, in kPa ml^{-1} min.

Venous pressure kPa	Basic saline	5% serum saline
zero	1.827 ± 0.083 (n = 50)	2.097 ± 0.447 (n = 8)
1.33	1.120 ± 0.057 (n = 50)	1.754 ± 0.092 (n = 36)

When the venous pressure was raised from zero to 1.33 kPa there was a significant drop in resistance of 38.68% ($P < 0.0001$). On addition of 5% human serum to the perfusate a resistance increase of 44.62% was recorded which is also significant at the $P < 0.0001$ level (Student's t-test). Baseline resistance was determined in eight experiments at zero venous pressure during perfusing with 5% serum saline.

When baseline resistance is plotted against weight of fish, an inverse relationship is seen (figure II.4). The equations of the lines fitted to the data are given with each figure. The relationships are similar to those found by Payan and Matty (1975) for perfused rainbow

FIGURE II.4a Weight versus perfused eel tail baseline resistance during perfusion with caudal venous pressure at 1.33 kPa and without serum in the perfusate. Error bars = ± 1 S.D. Fitted line has the equation

$$K_I(\text{kPa ml}^{-1}\text{min}) = 285\ \text{Wt(g)}^{-0.899}$$

F value for linearity of regression of transformed data = 31.7 for 1,48 d.f. ($F_{0.001\ [1,40]} = 12.6$)

FIGURE II.4b Weight versus perfused eel tail baseline resistance during perfusion with caudal venous pressure at zero and with 5% human serum added to the perfusate. Error bars = ± 1 S.D. Fitted line has the equation

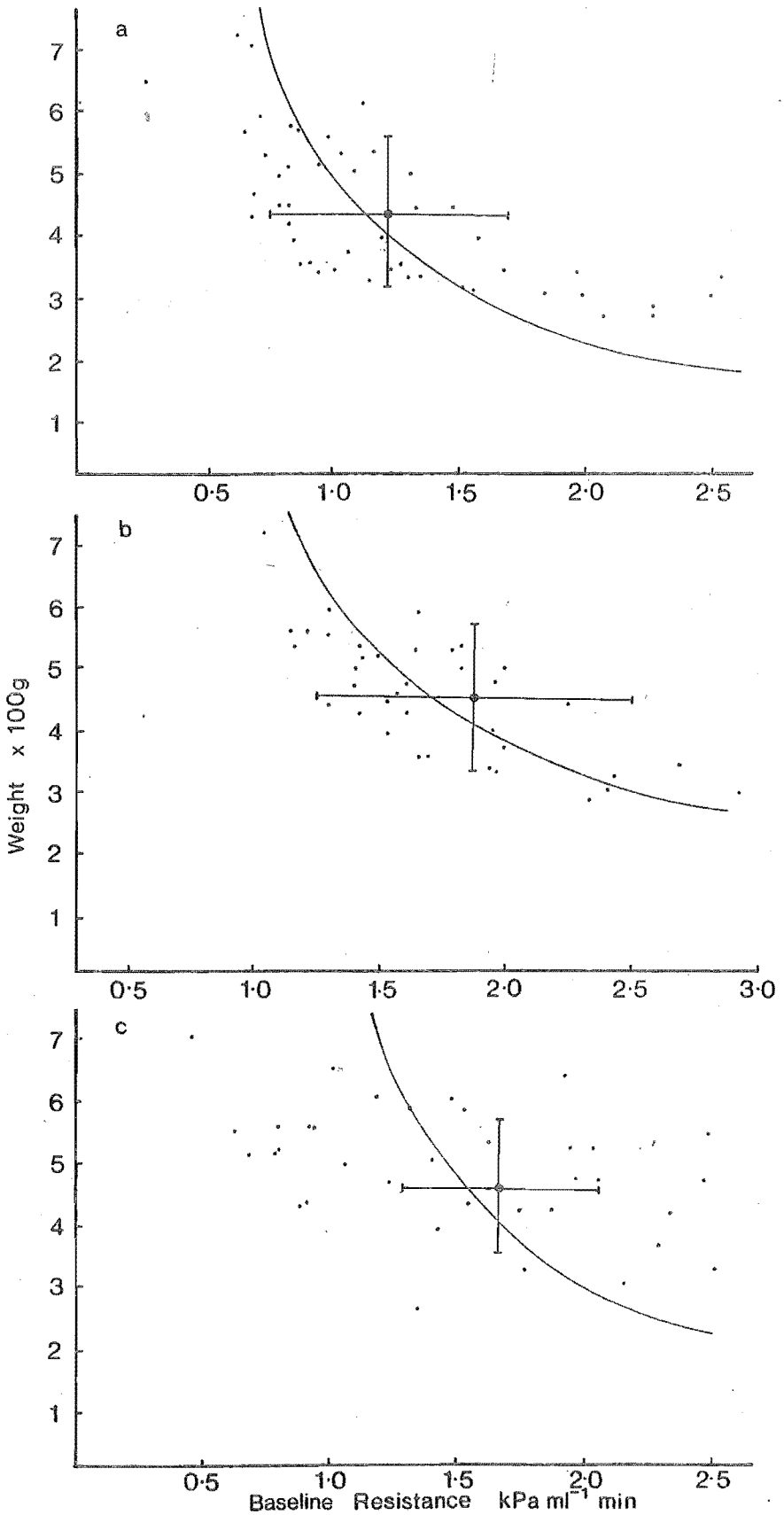
$$K_I(\text{kPa ml}^{-1}\text{min}) = 426\ \text{Wt(g)}^{-0.902}$$

F value for linearity of regression of transformed data = 47.6 for 1,43 d.f. ($F_{0.001\ [1,40]} = 12.6$)

FIGURE II.4c Weight versus perfused eel tail baseline resistance during perfusion with caudal venous pressure of 1.33 kPa and 5% human serum added to the perfusate. Greater scatter of points (see standard deviations) and a poorer fit of the line to the data indicates that resistance of preparations perfused with serum saline is less sensitive to weight. Error bars = ± 1 S.D. Fitted line is

$$K_I(\text{kPa ml}^{-1}\text{min}) = 68.7\ \text{Wt(g)}^{-0.619}$$

F value for linearity of regression of transformed data = 4.581 for 1,35 d.f. ($F_{0.025\ [1,35]} = 3.91$)


a
b
c
Weight x 100g
1
2
3
4
5
6
7
0·5
1·0
1·5
2·0
2·5
3·0
Baseline Resistance kPa ml^{-1} min

trout head preparations and those reported by Wood and Shelton (1975) for perfused trout trunks. The points on the graph for eel tails perfused with 5% serum saline have a greater scatter as indicated by the standard deviation. Although baseline resistance regressed against $\frac{1}{\text{weight}}$ produced significantly linear lines, better fits were obtained when logs were taken of both resistance and weight. Expression of resistance on a per weight basis would require different corrections for each set of perfusion conditions. Responses are expressed as either changes in resistance from baseline resistance (ΔK), or as percentage changes over baseline resistance ($\Delta K\%$). It is unclear whether or not ΔK or $\Delta K\%$ is inversely proportional to weight. Consequently no manipulation of the data to compensate for the weight of the tissue was used.

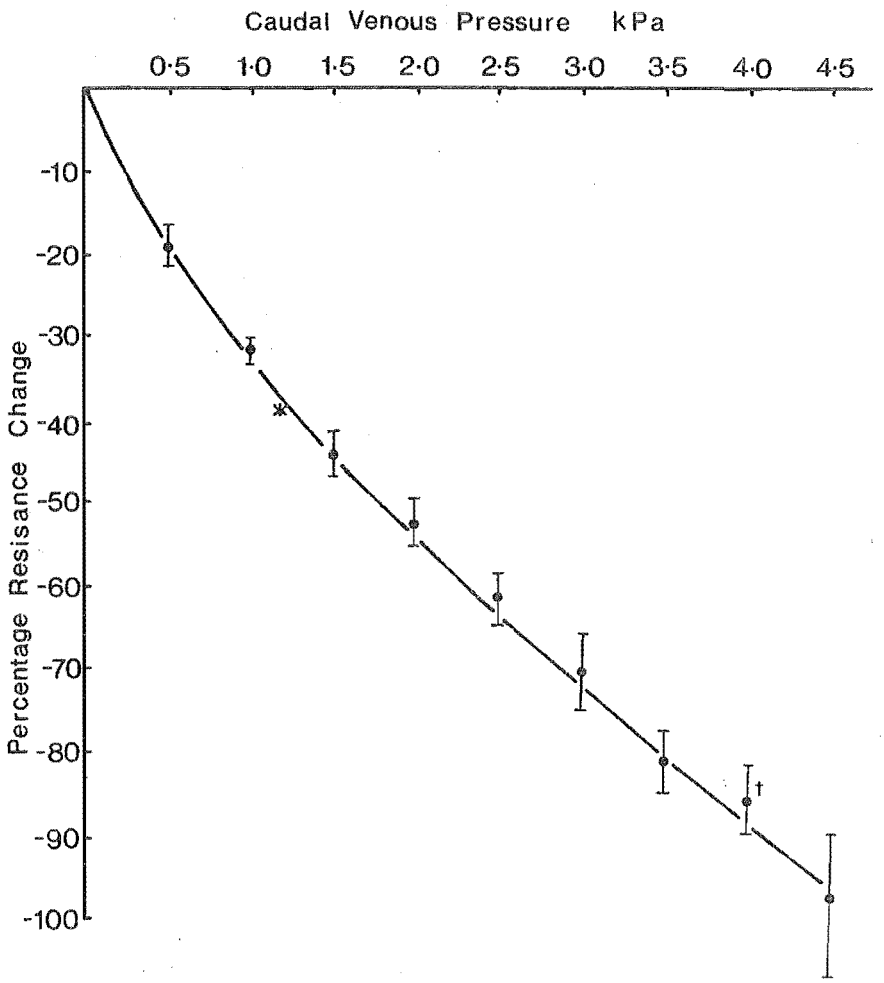
In eight preparations pump perfused with the basic saline the venous pressure was raised in 0.5 kPa steps from zero to 4.5 kPa. The results of these experiments are shown in figure II.5. As venous pressure was raised resistance fell by approximately 10% per 0.5 kPa. No myogenic increase in resistance was observed (see Johnson, 1977) and the vascular bed behaved to some degree as a set of distensible tubes (Folkow and Lofving, 1957). The change in resistance for the 50 tails perfused at a venous pressure of 1.33 kPa lies close to the curve. These results are similar to those reported by Wood (1974a) from isolated vertical tube perfused trout gills where the output pressure was raised from zero to around 3.9 kPa. When the venous pressure was raised above 3.5 kPa three of the eight perfused tail preparations exhibited zero outflow from the output cannula. Decreased outflow is to be expected during adjustment to increases in pressure since vascular volume increases (distensibility). Greater transmural pressure will result in more fluid passing into the tissues. At non-physiologically high venous pressures severe oedema could follow producing a short period of zero outflow. Very high venous pressures were maintained only until stable input and output pressures were attained, (one to two minutes) after which the venous pressure was lowered.

II.5.3 Saline experiments

II.5.3.1 Introduction and methods

As controls for the use of human serum in the perfusate, three tails were tested with a variety of salines. The resistance of the preparations were measured after ten minutes of perfusion with each of the salines and compared to the resistance with the basic saline. Adrenaline (AD) was

FIGURE II.5 Percentage changes in resistance in response to caudal venous pressure increments. Baseline resistance fell by approximately 10% per 0.5 kPa caudal venous pressure increment. The value for the percentage change in resistance after increasing venous pressure for all preparations perfused with the basic saline falls very close to the interpolated line (see *). Mean initial baseline resistance at zero venous pressure for preparations used in these experiments was 2.692 ± 0.0882 kPa $ml^{-1}min$ (n = 8). Error bars = ± 1 S.E.M. The symbol † indicates that three preparations showed zero outflow at this caudal venous pressure (for discussion see text). Two preparations only were subjected to caudal venous pressures of 4.5 kPa.


Caudal Venous Pressure kPa
0·5
1·0
1·5
2·0
2·5
3·0
3·5
4·0
4·5
Percentage Resisance Change
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
*
†

administered at three low doses, 50 pmoles, 100 pmoles and 200 pmoles in a bolus of 0.1 ml of the appropriate saline. Low doses were chosen so that the least possible desensitization with repeated administration would occur (Waud, 1968).

The salines were prepared as follows:

Salines 1 and 2. Twentyfive mls of human serum was thawed and placed into dialysis tubing, (Arthur Thomas, Philadelphia, No 4465 - A12) which permitted the movement of molecules of around 12000 molecular weight and less through the walls. The tubing was ligatured and placed into 30 mls of fresh water eel saline without PVP and stored at $4^{o}C$. After four hours the bathing saline was drained off and retained and the tubing washed twice in five mls of saline. The washings were retained with the bathing saline. This process was repeated twice more at eight and ten hours. After 12 hours of dialysis the contents of the tubing and the washings were reconstituted to make up separate salines containing 5% non diffusable serum proteins (tubing contents) and 5% dialysate.

Saline 3. Three $g.l^{-1}$ bovine serum albumin, (fraction V powder, Sigma Chemical Co.) in the basic saline.

Saline 4. 5% human serum in the basic saline.

Saline 5. Eel blood collected after heparinization (see section II.2.1) was centrifuged at 5000 rpm for ten minutes. The plasma was drawn off and added to the basic saline at a concentration of 5%.

Saline 6. Whole eel blood was used as the sixth perfusate in this series of experiments.

In all of the preparations serum subsamples were taken from the same original batch of serum. Preparation of the salines was repeated for each perfused tail preparation.

II.5.3.2 Results and discussion

The mean resistances for the preparations tested with the salines above are presented in table II.2. All of the test solutions increased resistance. The human serum protein saline increased resistance most and the bovine serum albumin the least.

Serum was dialysed to separate the high molecular weight proteins from the low molecular weight compounds and ions. Hargens et al. (1974) reported that fish proteins were retained across a dialysis tubing designed to retain compounds of 10,000 mwt or greater. Dialysis against the basic saline had the effect of only partially separating the two fractions. After the twelve hours and three changes of bathing saline the concentration of the low molecular weight fraction in the tubing is

Table II.2 Mean basline resistance (kPa ml^{-1} min) of three preparations perfused with a range of different salines.

	Normal saline	Serum proteins	Serum dialysate	3 gl^{-1} BSA	5% serum	5% Eel plasma
	0	1	2	3	4	5
Baseline resistance	0.7192	1.8728	1.0289	0.9044	1.7822	1.1647
± 1 S.E.	±0.0107	±0.0389	±0.01203	±0.0674	±0.0507	±0.0408

assumed to be reduced about eightfold. The results show that the high molecular weight fraction retained its activity and increased resistance above the level obtained by perfusing with 5% serum ringer. The low molecular weight fraction increased resistance to about half the resistance for perfusion with 5% eel plasma. This suggests that some low molecular weight substances in human serum have the ability to increase resistance. The resistance increase on perfusion with blood was 37.4% ± 9.8% (n = 5). It must be noted that most preparations tested with blood had undergone a full range of dilatory (3) or constrictory (1) responses to ISO or AD respectively. This treatment possibly lowered the reactivity of the vascular bed to perfusion with eel blood.

Responses of the preparations perfused with four of the test salines to low doses of adrenaline are presented in figure II.6 The greatest responses in absolute terms were found where serum proteins were present in the perfusate. When perfused with the basic saline, 3 gl^{-1} bovine serum albumin and 5% dialysate in the basic saline the responses to adrenaline were all very similar. If these results are expressed as percentage changes in resistance over baseline resistance, then the responses to each of these doses of adrenaline were similar for all the perfusates. This suggests that a higher baseline resistance results in a greater response to adrenaline as has been suggested by Wood (1974a).

The points to emerge from these controls are that the presence of serum in the ringer increases the vascular tone and the magnitude of the response to adrenaline of the isolated perfused eel tail. This tone is not caused by ions or low molecular weight (< 12000) compounds such as catecholamines in the serum. The responses to low doses of adrenaline were larger, but relative to baseline resistance were similar to the responses in the absence of serum. The possible mechanism by which serum proteins enhance responses to AD and other catecholamines was

FIGURE II.6 Mean resistance responses to 50, 100, and 200 pmoles adrenaline in three preparations perfused with four different salines. The enhanced responses when perfusate contains 5% human serum is partially due to elevated baseline resistance (for discussion see text).

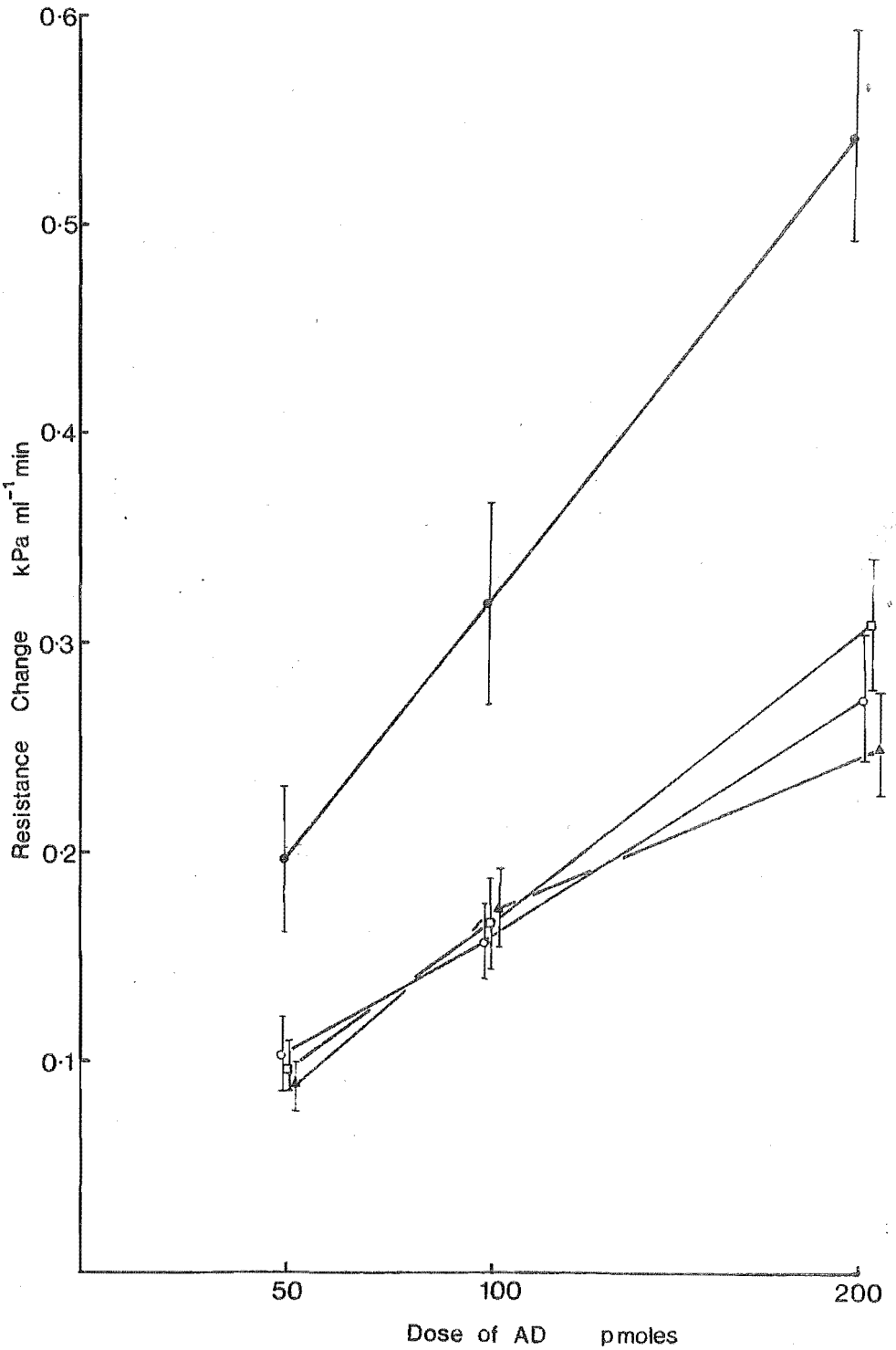
-□- Basic saline

-●- Basic saline plus 5% human serum 'proteins'

-○- Basic saline plus 5% 'dialysate' of human serum

-▲- Basic saline plus 3 $g.l^{-1}$ bovine serum albumin

Error bars are ± 1 S.E.M.


0·6
0·5
0·4
0·3
0·2
0·1
Resistance Change kPa ml^{-1} min
50
100
200
Dose of AD pmoles

discussed in section II.6.2.6. Potentiation of responses does not appear to be solely the result of elevated baseline resistance.

II.5.4 Flow versus pressure profiles

The vertical tube method of perfusion was used on 15 preparations. In each of the preparations at least two flow versus pressure differential (Q/Pd) profiles were constructed; one at a venous pressure of zero and the other at a venous pressure of 1.33 kPa. Nine of the 15 preparations were perfused with the basic saline, the other six had 3% serum added to the perfusate. Figure II.7 shows the curves for preparations without serum at zero and 1.33 kPa venous pressure. Figure II.8 shows the curves for tails perfused with 3% serum added at the same two venous pressures. Profiles were always determined after exsanguination and before any drugs had been administered. The profile at zero venous pressure was constructed first in all but two preparations. The two preparations where venous pressure was set to 1.33 kPa for the first profile produced results qualitatively the same as for the others.

The marked curvilinearity present in the Q/Pd profiles of trout gills (Wood, 1974a) is not seen in these curves. There is however, a slight convexity towards the Pd axis which indicates low vascular tone (Kuida, 1965). The progressive fall in resistance with time observed for the first 40 minutes by Wood (1974a) and Wood and Shelton (1975) was present in the perfused eel tail for only five to ten minutes. This initially high resistance at the start of perfusion is evident in the Q/Pd curves by downturn of the profiles at high venous pressure which corresponded with the start of perfusion. This effect was observed during both profiles where a period of very low flow intervened. It was however much smaller at the start of the second profile. The high initial resistance of the eel tail is the result of the near zero luminal pressure during preparation of the tail and after exsanguination which allowed the vessels to collapse. When the alternative perfusion system was connected and perfusion resumed, the vasculature was in a state of constriction which was rapidly dispelled in the presence of 'normal' luminal pressure. The bore of the tube used for the profiles was approximately twice the size of that used by Wood and Shelton (1975). Consequently there is a smaller change in flow rate per unit change in pressure. Thus at each point of the Q/Pd profile the conditions are more stable which may be a factor in reducing the 'viscous' effects which contribute to Q/Pd variability.

In the absence of serum, differences in flow at each pressure

FIGURE II.7 Flow versus pressure differential for preparations perfused by the vertical tube technique with the basic saline at caudal venous pressures of zero and 1.33 kPa. Flows are significantly different at all pressure differentials at the $P < 0.005$ level (Student's t-test, $n = 9$). At perfusion pressures above 8.0 kPa there was a convexity toward the pressure axis particularly in preparations perfused at zero venous pressure. This is interpreted as passive distension of the vessels at the start of perfusion after periods of very low flow when vessels would collapse. Lack of convexity over the rest of the pressure range suggests that there is significant resting tone (Kuida, 1965). Error bars are ± 1 S.E.M.

-O- caudal venous pressure = 1.33 kPa

-●- caudal venous pressure = zero

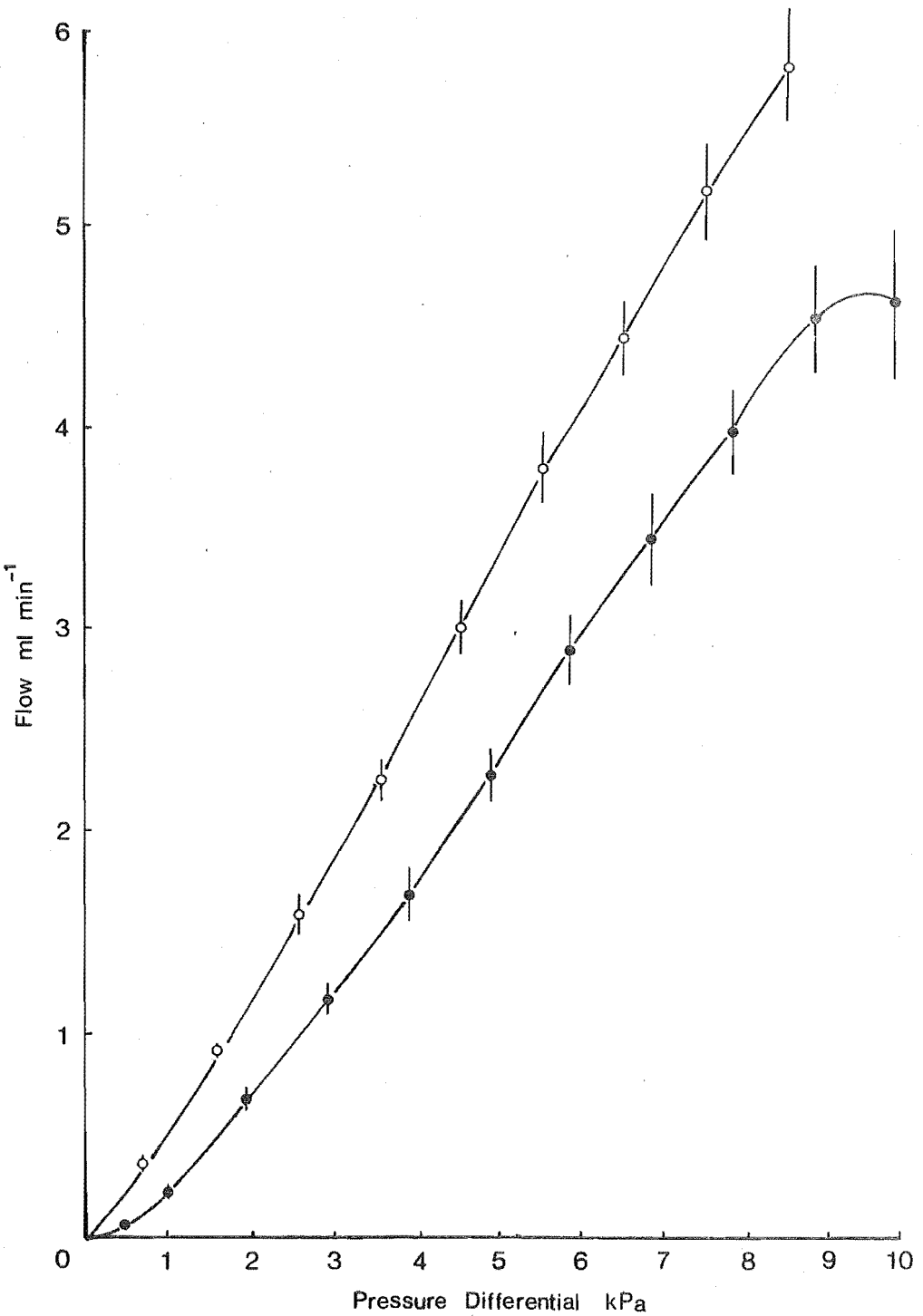
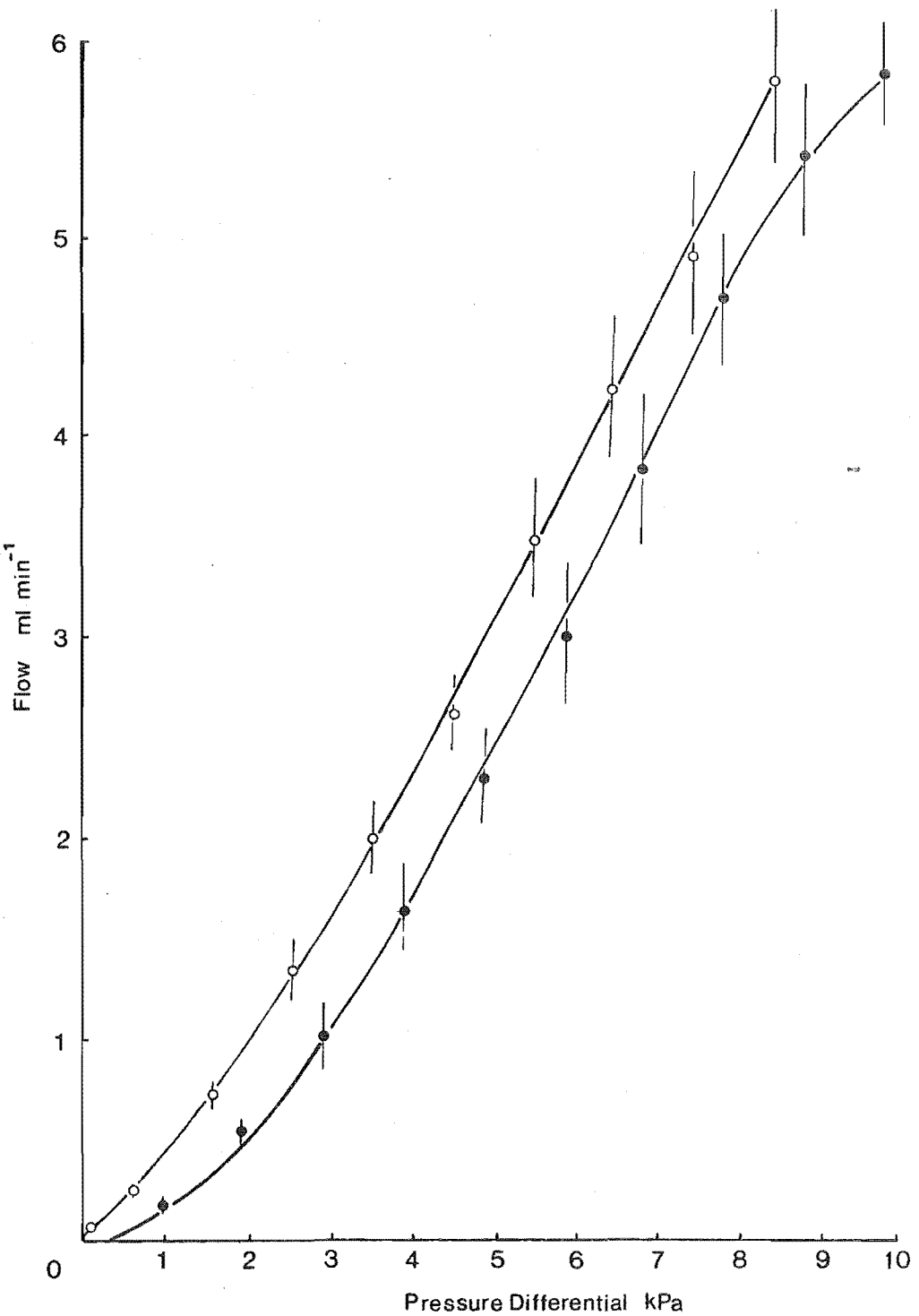

6
5
4
3
2
1
0
Flow ml min^{-1}
1
2
3
4
5
6
7
8
9
10
Pressure Differential kPa

FIGURE II.8 Flow versus pressure differential for six preparations perfused by the vertical tube technique with 5% human serum in the perfusate at caudal venous pressures of zero and 1.33 kPa. There was no significant differences between flows at any pressure differentials. Greater variation of flow values at each pressure differential was typical for preparations perfused with serum saline. Slight convexity toward the flow axis suggests that resting vascular tone was greater in preparations perfused with serum saline than in those perfused with the basic saline. Error bars are ± 1 S.E.M.

-O- caudal venous pressure = 1.33 kPa

-●- caudal venous pressure = zero


6
5
4
3
2
1
0
Flow ml min^{-1}
1
2
3
4
5
6
7
8
9
10
Pressure Differential kPa

differential between preparations perfused at venous pressures of zero and 1.33 kPa were all highly significant ($P < 0.005$-0.001; Student's t-test). In the presence of serum none of the differences in flow at any pressure differential was significant. Addition of 3% human serum to the perfusate resulted in smaller changes in resistance to flow when venous pressure was raised.

Resistance calculated from vertical tube perfusion at the flow rate later used for pump perfusion of the same preparations, and baseline resistance for pump perfusion were not significantly different. Without serum in the perfusate vertical tube resistance at equivalent flows and caudal venous pressures of 1.33 kPa were lower than pump perfusion baseline resistances under the same conditions (Q/Pd K = 1.226 ± 0.1357 kPa ml^{-1} min; pump K = 1.751 ± 0.2143 kPa ml^{-1} min; n = 9). When 3% human serum was added to the perfusate, vertical tube perfusion resistance was higher than pump perfusion baseline resistance at equivalent flows and caudal venous pressures of 1.33 kPa (Q/Pd K = 2.2799 ± 0.1327 kPa ml^{-1} min; pump K = 2.105 ± 0.09673 kPa ml^{-1} min; n = 6), and differences were smaller. Addition of 3% human serum to the perfusate reduced the effects of different perfusion methods upon estimates of vascular resistance in this preparation. Addition of serum to the perfusate has imparted a degree of vascular autoregulation to this preparation that was otherwise absent.

II.5.5 X-ray study of the preparation

II.5.5.1 Introduction and methods

One question that remained unanswered was how well perfused was the preparation. As the time course of the reactions of the vasculature and lymph heart is dependent upon the amount of drug presented to appropriate area of the preparation, then this question seemed to be important.

To study this, one perfused preparation was prepared as normal at the University of Canterbury and subsequently transported to the Princess Margaret Hospital, Christchurch. During transport, perfusion was maintained by a constant pressure head (~ 3.0 kPa), and the preparation was covered with saline. Once at the Radiology Department of the Hospital the tail was placed in a shallow plastic tray and covered with paper towels soaked in saline. The pump replaced the pressure head and the venous pressure was raised to 1.33 kPa.

Radio-opaque medium 'Conray 280' (35% w/v meglumine bothalamate),

was used at full strength and was either injected into the perfusion line, (0.5 ml) over a period of 30 s or was introduced by perfusion (0.5 ml) taking approximately 23 s. Smaller doses and dilutions of the medium proved insufficient to show up any but the largest vessels. The time that elapsed between the start of perfusion and taking the first plate was around 100 minutes. Although temperature was not controlled, the day was cool, (10-15^{o}C). X-ray plates were taken at 30 s intervals for up to 240 s (see Mott, 1950b).

II.5.5.2 Results and discussion

Of the six trials, only two yielded satisfactory results. The four unsuccessful attempts were so because insufficient contrast medium was used. The two successful sets were taken with the venus pressure at zero and 1.33 kPa. The former, at venous pressure at zero, received the medium by injection, (I); the latter received the medium by infusion into the perfusion line, (II). The first plate from trial II showed the medium just entering the caudal artery. This indicates that much of the first 30 s after the medium passed the depulsator was taken up by filling the dead space. Thus this point is very near time zero when compared to I. Injection of 0.5 ml over 30 s is equivalent to increasing the flow rate through the tail by ~ 40%. Thus we could expect more rapid filling of the vessels in trial I than in trial II. This in fact was the case.

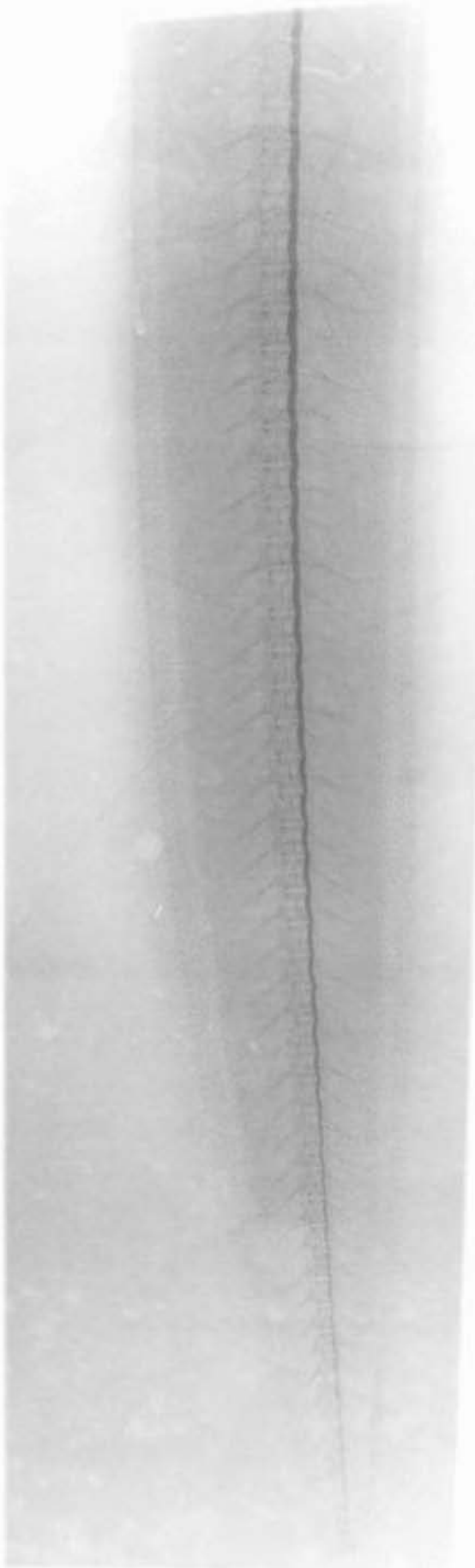
Trial I. Thirty seconds after the start of injection, the first plate reveals that all but the last 30 mm of caudal artery was filled with contrast medium and that the segmental arteries were filled or filling. At 60s, both the caudal artery and caudal vein contained medium, the artery showing up more densely. The vessels in the tip of the tail were most clearly seen at this time and the segmental arteries still contained some medium. By 90 s, the caudal artery was empty and the density of medium in the caudal vein was decreasing. At 120 s, only the most anterior part of the caudal vein contained appreciable amounts of medium. After the next 30 s had elapsed, at time 150 s, only a diffuse 'glow' could be seen in the caudal vein. Thus it is apparent that a large bolus injected into the perfusion line enters all arteries within 60 s. Vessels of the lymph heart area are filled with the bolus fluid within 60 s. Any agent in such a bolus would be in contact with lymph heart muscle within 60 s.

Trial II. The first plate taken at thirty seconds after the medium passed through the depulsator is taken as time zero. Within 30 s all but the last 25 mm of caudal artery was clearly filled along with the

anterior segmental arteries. At time 60 s the caudal artery was completely filled even to the most caudal parts, (see figure II.9). By 90 s medium was in both the caudal artery and vein. The density of the medium in the caudal part of the tail is greater in the artery than in the vein. In the cephalic part of the tail, the density was greater in the vein than the artery. This clearly indicates that the medium was well distributed throughout the vascular bed after 90 s. By 120 s both the caudal artery and caudal vein were clearing. The caudal 50 mm of the caudal artery still seemed to contain a fair concentration of medium. At 150 s the vein and artery are indistinguishable and the whole tail has a diffuse radio-opacity.

Although the arterial filling is more rapid in the case of an injected bolus, the circulation time through the perfused eel tail is of the order of 150 s. The arteries including the resistance vessels are filled within the first minute while the veins are filled within 120 s. The results also show that there were no parts of the tissue that received little or no perfusate. The tissue is well perfused and has a circulation time of 60 to 90 s. The estimated circulation time of rainbow trout is around 60 s (Davis, 1970).

FIGURE II.9 Photograph from an X-ray plate 60 s after infusion of 0.5 ml of radiopaque medium into the caudal artery. The caudal artery appears completely filled as are most of the segmental arteries.



II.6 Vasoactive Drugs and Vascular Resistance

II.6.1 Introduction

Vascular tone is controlled by three principal mechanisms; the autonomic nervous system (Folkow, 1960; Mellander and Johansson, 1968; Burnstock, 1969): circulating vasoactive hormones, (Kirby and Burnstock, 1969; Burnstock, 1969): and localised myogenic and metabolic factors (Johnson, 1977). Blood borne catecholamines appear to play a major role in fish circulatory regulation (Randall and Stvens, 1967; Burnstock, 1969; Campbell, 1970; Wood and Shelton, 1975; Capra and Satchell, 1977c). Burnstock (1969) suggested that during vertebrate evolution the autonomic nervous system assumed a greater degree of control over vascular functions and that circulating catecholamines represent a more primitive type of control system. The two naturally occurring catecholamines identified in fish, adrenaline and noradrenaline, are both capable of stimulating alpha and beta adrenergic receptors. The alpha stimulating potency of adrenaline (AD) is greater than that of noradrenaline (NAD), which is in turn much greater than that of the synthetic beta agonist isoprenaline, (ISO); AD > NAD >> ISO. For $beta_1$ receptors, ISO >> NAD > AD and $beta_2$ receptors, ISO >> AD > NAD, (Furchgott, 1967). Because adrenergic receptors are defined as alpha or beta according to the potency of the drugs above the only way to define an adrenergic receptor in a new preparation is by potency comparisons (Arnold, 1972; Wood and Shelton, 1975). The use of selective alpha or beta antagonists often provides additional support for one or other type of receptor but cannot strictly be taken alone to delineate between adrenergic receptor types.

Teleosts show increased plasma adrenaline and noradrenaline concentrations in response to stressful situations. Nakano and Tomlinson (1967) stressed rainbow trout by tail grasping and reported an increase in adrenaline concentration from 2.3×10^{-8}M at rest to 7.2×10^{-7}M after stress. Noradrenaline concentration changed under this stress from 1.7×10^{-8}M to 1.4×10^{-7}M. Mazeaud, Mazeaud and Donaldson (1972) exposed three species of salmon, the coho (*Oncorhynchus kisutch*), sockeye (*O. nerka*), and chinook (*O. tshawytscha*) to a variety of stimuli including struggling in air, hypoxia and thermal stress. These authors reported similar increases in plasma catecholamine concentrations for all stimuli. Adrenaline concentration rose from 3.5×10^{-8}M to 7.6×10^{-7}M, and noradrenaline rose from 6.2×10^{-9}M to 1.6×10^{-7}M. The cod

showed plasma adrenaline concentration increases from 4.4 x 10^{-8}M to 2.3 x 10^{-7}M and noradrenaline concentration increases from 5.9 x 10^{-9}M to 6.2 x 10^{-8}M when exposed to air (Nilsson, Abramsson and Grove, 1976). Recently Butler, Taylor, Capra and Davison, (1978) have reported similar changes in catecholamine concentrations in the dogfish (*Scyliorhinus canicula*) during hypoxia. All of these reports show consistent increases in plasma catecholamine concentrations of around 15 times during stress.

Despite the problems in assay the reported catecholamine concentration changes are able to explain changes in gill circulatory behaviour *in vivo*, and allow calculations to be made which compare well with results from isolated perfused gill preparations (Wood, 1974a; Payan and Girard, 1977).

The compatability of these sets of results from gills contrasts sharply with similar comparisons made with results from the systemic vasculature of teleosts. Data to date suggest that the predominant response of the systemic vasculature to catecholamines is an increase in resistance (see Wood, 1976). During swimming when catecholamine concentrations could be expected to rise, a fall in systemic resistance has been reported (see Kiceniuk and Jones, 1977). Increased cardiac output which could account for much of the resistance change has been postulated to be the result of either a decrease in vagal inhibition (Randall, 1966) or to excitatory adrenergic innervation, (Gannon, 1971; Holmgren, 1977). Circulating catecholamines appear to play a minor role in regulation of teleost cardiac output.

Responses to exogenous catecholamines may reflect the effects of these drugs upon receptors which normally receive AD or NAD from adrenergic nerve terminals. Whatever the natural mode of delivery of catecholamines to the gill vasculature, responses are in accord with *in vivo* results. In the absence of well defined decreased systemic resistance responses to catecholamines from isolated preparations it is difficult to explain the rather sparse *in vivo* results which indicate that resistance across the systemic vascular beds falls when blood catecholamine concentrations are naturally elevated (Kiceniuk and Jones, 1977).

Studies on the systemic adrenergic system of fish are numerous. Within the teleosts three groups have received particular attention, namely trout, cod and eels. All reports show the presence of alpha adrenoreceptors. Studies that provide evidence for alpha receptors only include those by Keys and Bateman (1932); Mott (1951); Fange (1953); Stevens and Randall (1967a); Johansen and Reite (1968);

Reite (1969); Stevens, Bennion and Randall (1972); Nilsson (1972); Holmgren and Nilsson (1974); Forster (1976a). Since Chan (1967) provided evidence for beta adrenoreceptors in the systemic vasculature of the european eel, there have been a growing number of reports which support their presence in fish systemic vessels: Chan (1969); Stray-Pedersen (1970); Gras, Perrier, Perrier and Gudefin (1971); Helgason and Nilsson (1973); Holmgren and Nilsson (1974); Capra (1975); Chan and Chow (1976); Wood (1976); Capra and Satchell (1977a,c); Holmgren (1978). Apart from Gras et al. (1971) whose study was primarily on extracellular space in muscle slices, beta dilation in the trout systemic vasculature has been very difficult to demonstrate (Wood, 1976). Only against adrenergic tone produced by adrenaline could Wood elicit a beta dilatory response. Wood (1976) also made the observation that d-isoproterenol has alpha antagonistic effects. He mentioned that previous studies often used the d-, l- raecemic mixture and could cast doubt on whether the dilation observed was beta mediated or simply antagonism of an already present alpha tone by the d-optical isomer. This study of the perfused eel tail has used the laevo rotatory optical isomer of isoproterenol exclusively, which has virtually no alpha antagonistic activity, and is the active beta agonist in the raecemate (Jenkinson, 1973). Many of the above-mentioned studies used isolated organs or vessels (coeliac artery, swimbladder, spleen) which may only play a small part in the natural decreases in systemic resistance. Thus a beta dilatory response should not be expected from all the variety of tissues within the fish trunk. Furthermore in the absence of any vascular tone (see Wood and Shelton, 1975; Wood, 1976) it would be very difficult to produce a dilatory response to any stimulus.

Autonomic control of fish vasculature has been suggested to be totally lacking or to be only rudimentary (Burnstock, 1969). Recent work by Holmgren (1978) and Smith (1978) suggests that it may be better developed than was previously thought. The effects of metabolites and local changes in pH may well play an important role in systemic vascular resistance control especially if flow through active tissues (e.g. muscles) is reduced (Johnson, 1977). Lastly we should bear in mind that catecholamines not only effect vascular tone but also modify extravascular space (Gras et al., 1971; Pic, Mayer-Gostan and Maetz, 1974) and levels of free fatty acids and glucose in the blood (Mazzeaud, 1972; Mazzeaud et al., 1977). Liberation of metabolic substrates and their movement through extravascular space may well be an important function of catecholamines during exercise.

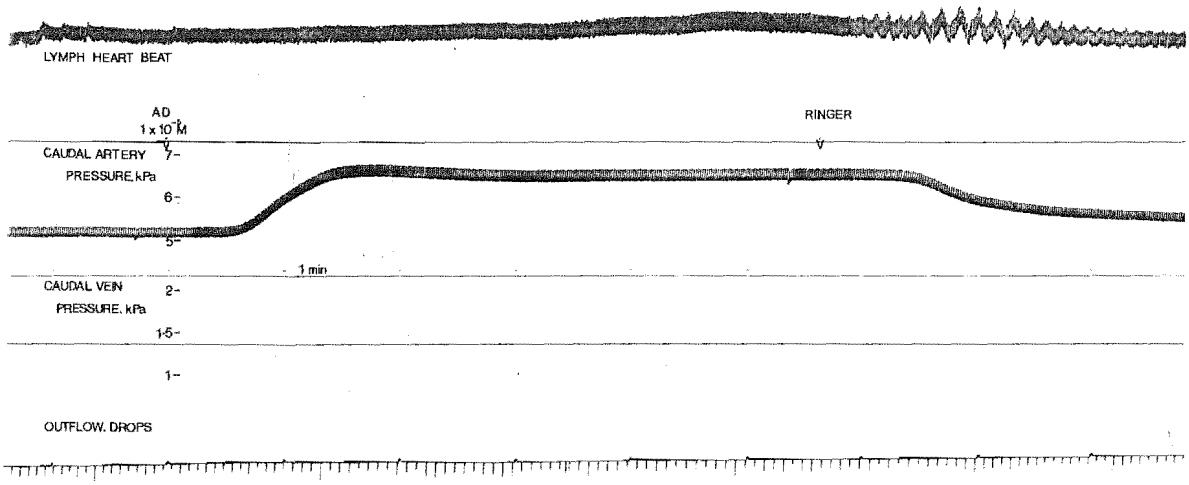
II.6.2 Dose response curves for adrenaline, noradrenaline and isoprenaline

II.6.2.1 General Results

Cumulative dose response curves were prepared for three sympathetomimetic drugs, adrenaline, (AD), noradrenaline, (NAD), and isoprenaline, (ISO). All doses are expressed as molar concentrations in the perfusate, or as moles of drug given as a bolus. Response is expressed either as change in resistance, (ΔK) in units of kPa ml^{-1}min or as the percentage change in resistance over baseline resistance, ($\Delta K\%$). Adrenaline and noradrenaline both caused increases in resistance, Isoprenaline caused decreases in resistance at concentrations below 1 x 10^{-4}M. Threshold concentrations for AD and NAD were between 1 x 10^{-10} and 1 x 10^{-9}M. The threshold for ISO was between 1 x $10^{-11}$and 1 x 10^{-10}M. All data from which the curves were constructed are tabulated in appendixes A.1 - A.20. It will be seen that not every preparation was tested at each concentration. In some cases a dose near that required to produce a half maximal response, (ED50) was administered without preceding doses (see appendix A.3 for AD). The effect of this method was to remove any desensitization of the receptors by previous administrations (Waud, 1968). The randomization test for two independent samples was applied to the data from the responses of the preparations to 5 x 10^{-7}M AD (Siegel, 1956). This test revealed that there was no significant difference between responses from preparations that had been exposed to a full series of AD and those that received the same dose without previous drug administrations ($P > 0.1$). There is no significant desensitization of the receptors during the construction of AD cumulative dose response curves in this preparation.

It was not possible to prepare full dose response curves for the bolus method since doses of 10 and 100 n moles of all drugs often initiated violent muscular contractions of the tail suspended in the bath. Skeletal muscle contraction in response to high concentrations of catecholamines was noted throughout this study. Occasionally swimming movements were apparent (see figure II.10) especially when drugs were administered via the perfusion line. Wood and Shelton (1975) also noted this in the trout trunk but were unable to offer an explanation. Catecholamines can elevate the general level of excitability of the mammalian central nervous system and could offer an explanation in terms of spontaneous motor discharges (see Vogt, 1973). Two preparations which failed to give muscular contractions in response to 100 nmoles ISO were given 1 µmole doses of the drug. This caused muscular contractions.

FIGURE II.10 Record of a response to infusion of 1 x 10^{-4}M adrenaline in which at the end of the ten minute period of infusion regular flexions of the tail were recorded as deflections of the mechanotransducer rested against the lymph heart. The tip of the tail was pinned to the wax block to facilitate lymph heart records, however when particularly violent contractions of the tail occurred the tail was pulled from the block giving a spurt of outflow from the venous cannula as it contracted. This swimming response was only observed in preparations where large doses of sympathetomimetic agents were administered. Mean frequency of tail flexion was 8.25 ± 0.24 cycles per minute (n = 17). Swimming at 15 cm s^{-1} was accomplished by live intact unanaesthetised eels at a tail flexion frequency of about 25 cycles per minute. Preparation number A203. Tail weight = 229 g. Tail resistance change = +129.87%.


LYMPH HEART BEAT
AD
1 x 10⁻⁵M
RINGER
CAUDAL ARTERY
PRESSURE, kPa
7
6
5
1 min
CAUDAL VEIN
PRESSURE, kPa
2
1·5
1
OUTFLOW, DROPS

The resistance responses were in agreement with an extrapolation of the dose response curves for these preparations (see appendix A.17 and A.18).

II.6.2.2 Analysis of dose response curves prepared from responses to infused drugs

Full dose response curves are often sigmoid in shape (Arunlakshana and Schild, 1959; Waud, 1968). In trying to describe the response in terms of the amount of drug administered, strictly speaking, nothing should be assumed about the relationship. However in the light of the above information, from experience with similar experiments (Holmgren and Nilsson, 1974; Wood and Shelton, 1975) and from qualitative assessment of the results to be described here, the curves were assumed to be sigmoid.

For the simplest model of drug receptor interaction the following equation is adequate,

$$A + R \rightleftharpoons AR \rightarrow E \qquad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad 1$$

where A is the drug,
R is the receptor, and
E is the response.

The response can be described by the logistic equation

$$E = M \frac{Y^p}{Y^p + K^p} \qquad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad 2$$

where M is the maximum response
p reflects the slope of the curve
Y is the fractional receptor occupancy, and
K is an empirical location constant

(Waud, 1968, 1975; Volund, 1978). The tissue under observation in this study however contains more than one receptor. The facts that AD and NAD caused increased vascular resistance and ISO caused decreased resistance to flow suggested the presence of both alpha and beta receptors. Thus description of the response in terms of one set of receptors is not applicable. An empirical approach to the description of the dose response curve was therefore used. A logistic equation was chosen because it provides a good fit, is reasonably flexible and is easily calculated. The form of the equation contains elements of the curve which provide useful information about the response and is as follows,

$$E = M \frac{A^p}{A^p + K^p} \qquad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad 3$$

where E is the response
M is the maximum response
A is the concentration of drug
K is the ED50, and
p reflects the slope of the curve

This equation is derived from that given by Waud (1975) to describe sigmoid dose response curves in the presence of an antagonist. When zero antagonist is substituted throughout equation 52 of Waud (1975) equation three results. Curve fitting was performed by an iterative process using the least squares process. The values of M, p, and K were allowed to vary until the best fit was found, as measured by the sum of the squares of the differences between observed and estimated responses. The search procedure for the parameter values is that of Powell (1964). Mean responses for each drug concentration were calculated and used to fit the model (see Volund, 1978). Since responses are measured as differences of resistance from baseline resistance, all data points have equal weights. In addition to these points the curves were constrained to pass through the origin. It is logical to expect zero response when no drug is administered. The dose and the response at the origin were set to very small values and given weights of 100 times the weight of the other points.

Curves fitted to the data had multiple correlation coefficients of greater than 0.97 (0.988 ± 0.0046, mean $\pm$ 1 S.E.M.; $n = 10$) indicating the propriety of the model for these data.

Comparisons of the response to the same drug under different perfusion conditions and comparisons of responses to different drugs under the same conditions are facilitated when estimates of parameters such as ED50, maximum response and slope are derived from all the information simultaneously. Tests to determine the significance of differences between the nonlinear parameters slope and ED50 are not possible with this method. Similarly analysis of covariance of the regression lines is not possible because the regression is nonlinear and the search procedures may have lead to biased estimates of the parameters. However, the standard error of the response estimate, when multiplied by 1.96 gives the 95% confidence limits and allows the comparison of responses to equal doses from two fitted curves. Should the point on one curve fall outside the limits of the other, then at that point there is deemed to be a significant difference between responses. Confidence limits for dose, the independent variable, were taken as horizontal excursions within the 95% response confidence limits. Data from the dose response curves are presented in table II.3.

Table II.3 Summary of results from perfusion line administration dose response curves.

Drug	ΔK/ΔK%	Serum/ No serum	Maximum	ED50 95% confidence limits	Slope	Calc. response at 1 x 10^{-5}M	Time to peak s	S.E. of response estimate
AD	ΔK	No serum	2.640	5.152×10^{-7} $4.16 - 6.02 \times 10^{-7}$	9.935	1.703	176.4	±0.048
	ΔK%	No serum	287.170	2.602×10^{-7} $1.78 - 3.09 \times 10^{-7}$	13.297	241.798	±35.4	±2.921
	ΔK	Serum	3.285	5.157×10^{-7} $3.71 - 7.58 \times 10^{-7}$	6.733	2.224	162.3	±0.032
	ΔK%	Serum	225.966	3.042×10^{-7} $1.99 - 3.24 \times 10^{-7}$	10.577	184.967	±31.1	±2.286
NAD	ΔK	No serum	2.942	5.887×10^{-7} $1.12 - 6.30 \times 10^{-7}$	6.172	1.691	241.9	±0.029
	ΔK%	No serum	285.280	1.348×10^{-6} $1.00 - 1.90 \times 10^{-6}$	4.984	133.900	±76.2	±4.984
ISO	ΔK	No serum	-0.171	1.218×10^{-8} $1.04 - 1.44 \times 10^{-8}$	5.776	-0.0786	285.0	±0.0045
	ΔK%	No serum	-18.760	8.549×10^{-9} $6.30 - 11.21 \times 10^{-9}$	5.878	-10.021	±72.5	±0.578
	ΔK	Serum	-0.367	9.079×10^{-9} $6.76 - 12.58 \times 10^{-9}$	5.949	-0.1918	252.6	±0.0128
	ΔK%	Serum	-22.018	8.194×10^{-9} $5.12 - 12.02 \times 10^{-9}$	6.584	-12.137	±36.1	±1.162

ED50 ratios AD : NAD

ΔK 1.00 : 1.141

ΔK% 1.00 : 4.431

Potency ratio at 1 x 10^{-5}M AD : NAD

K 1.00 : 1.040

K 1.00 : 0.723

Resistance in kPa ml^{-1}min, concentrations in moles l^{-1}, time in s.

II.6.2.3 Infusion dose response curves for AD

The dose response curves for adrenaline were consistently steeper than those for NAD or ISO (see table II.3; figure II.11). The dose required to produce a half maximal response (ED50), occurs at the point of inflection where the slope is steepest. Small changes in dose around this point results in larger changes in response than at any other point on the curve. The ED50s for the four AD curves are between 2.6 and 5.2 x 10^{-7}M. Perfusion of 1 x 10^{-10}M and 1 x 10^{-9}M AD resulted in dilatory responses in two preparations (see appendix A.3).

Addition of 5% human serum to the perfusate elevated baseline resistance (see section II.5.2). When AD was administered to serum treated preparations the following changes were noted.

1. The slope of the curves was depressed.

2. The maximum absolute change in resistance (ΔK), was significantly larger.

3. The maximum change in resistance relative to the baseline resistance (ΔK%), was significantly smaller.

4. The ED50 estimates from either the ΔK or ΔK% curves were unchanged by the presence of serum.

II.6.2.4 Infusion dose response curves for NAD

The dose versus absolute response curve for NAD administered by perfusion line has a ED50 of 5.8 x 10^{-7}M, a slope of 6.17 and a maximum response of 2.94 kPa ml^{-1}min. The maximum was greater than that for AD under identical perfusion conditions; that is without serum. From the curve of dose versus ΔK% the ED50 was 1.3 x 10^{-6}, the slope was 4.98 and the maximum response was +285.28% (see figure II.12). The ED50 of this curve was the greatest for any drug under any conditions tested and the slope the lowest. The maximum percentage change in resistance is very similar to that obtained for AD without serum.

The ratios of the doses that produce half maximal responses are given in table II.3. When the ED50s of AD and NAD without serum are compared, AD is found to be more potent giving dose ratios of between 1.00 : 1.14 (ΔK) and 1.00 : 4.43 (ΔK%). When the absolute responses to the same molar concentrations of the two drugs are compared (1 x 10^{-5}M) they are found to be almost equipotent, (AD : NAD, 1.00 : 1.04). When ΔK% is compared in this manner AD gives a greater response (1.00 : 0.723), AD : NAD.

FIGURE II.11 Dose response curves constructed from peak response to infusion of 1×10^{-11}M to 1×10^{-3}M adrenaline. Data from these curves are summarised in table II.3. All responses were recorded while perfusing at caudal venous pressures of 1.33 kPa. Stippled area indicates the 95% confidence limits of the response estimates.

a. Responses expressed as kPa $ml^{-1}min$. No serum in the perfusate.

b. Responses expressed as % changes in resistance over baseline resistance, $\Delta K\%$. No serum in the perfusate.

c. Responses expressed as kPa $ml^{-1}min$. 5% human serum added to the perfusate.

d. Response expressed as % change in resistance over baseline resistance. 5% human serum added to the perfusate.

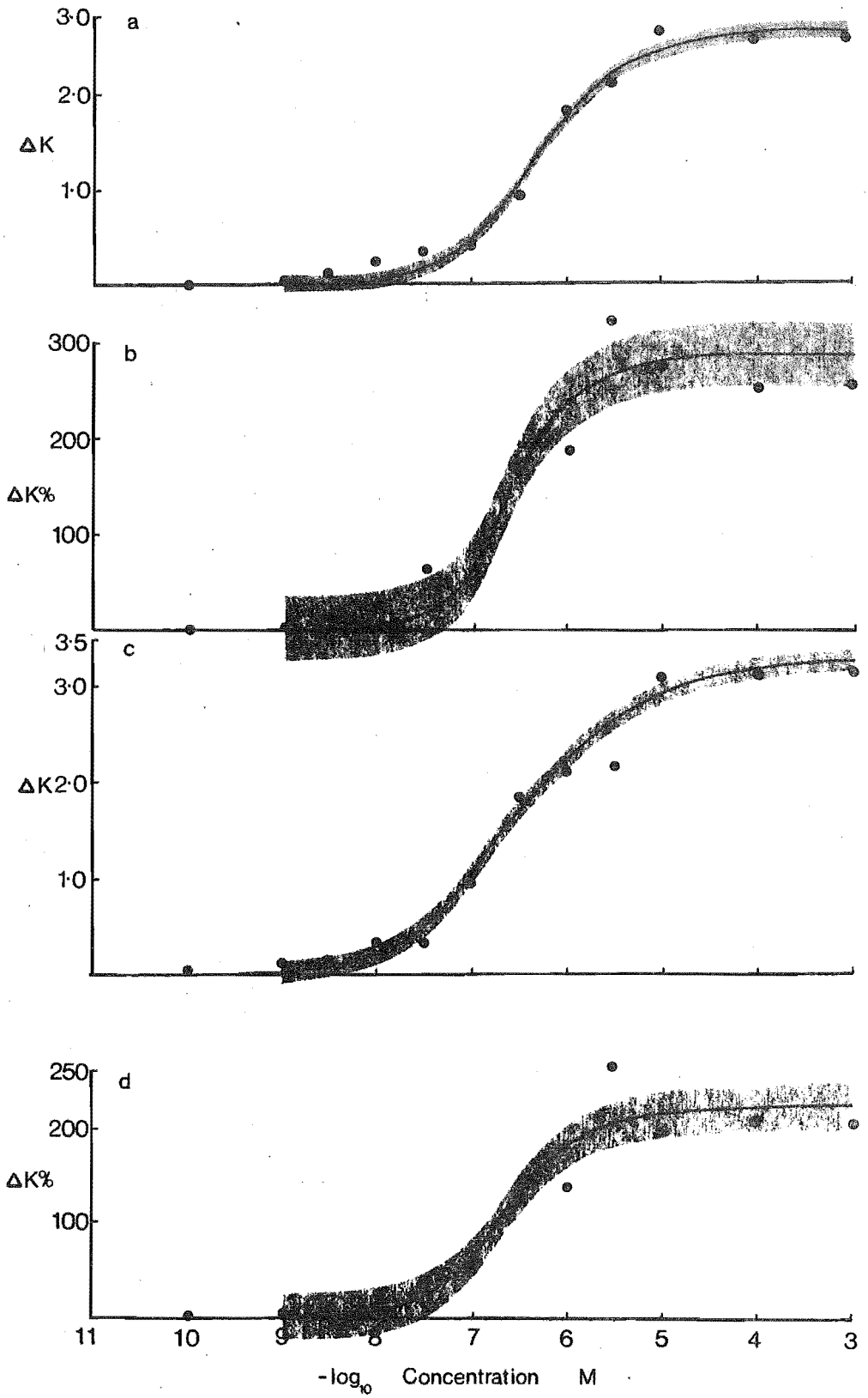
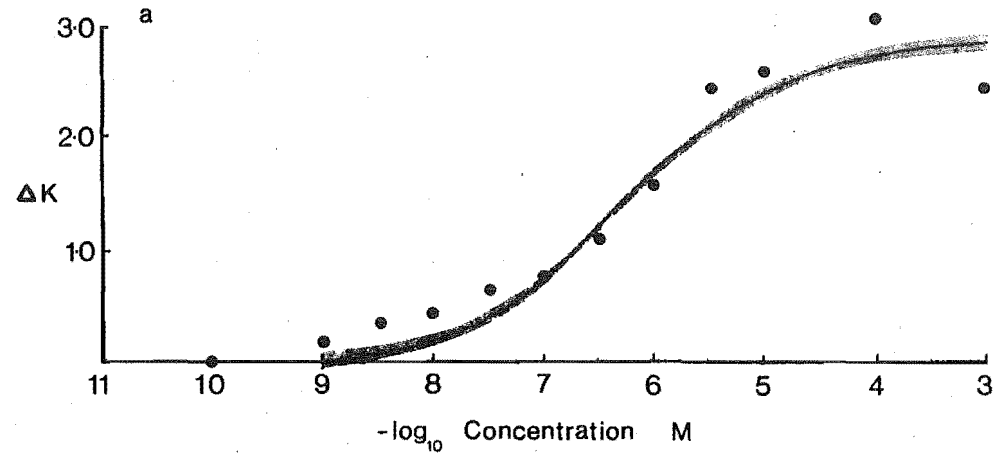
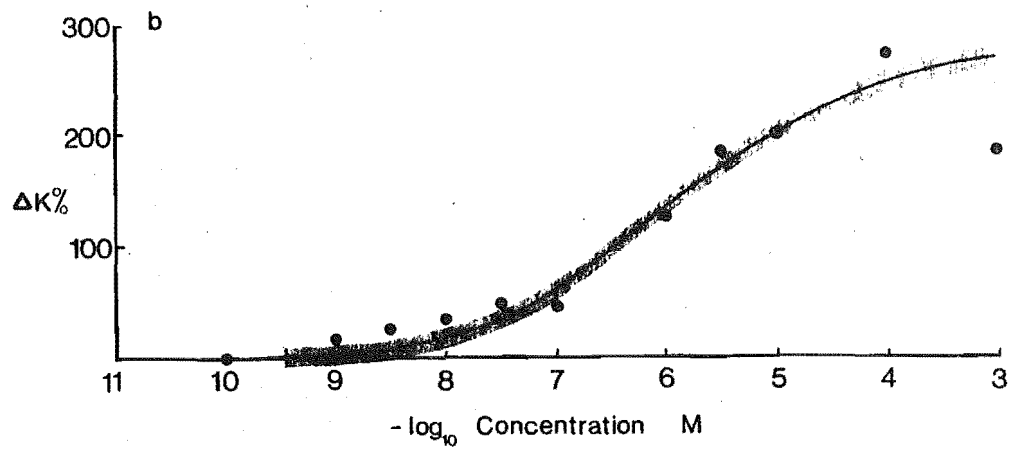

a
3·0
2·0
ΔK
1·0
b
300
200
ΔK%
100
c
3·5
3·0
ΔK2·0
1·0
d
250
200
ΔK%
100
11
10
9
8
7
6
5
4
3
-log10 Concentration M

FIGURE II.12 Dose response curves constructed from peak responses to infusion of 1 x 10^{-11}M to 1 x 10^{-3}M noradrenaline. Data from these curves are summarised in table II.3. All responses were recorded while perfusing at caudal venous pressures of 1.33 kPa and without serum in the perfusate. Note that the responses to 1 x 10^{-3}M noradrenaline were smaller than to 1 x 10^{-4}M. This was not observed in the analogous experiments using adrenaline and is interpreted as stimulation of beta two dilatory adrenergic receptors only at very high doses of noradrenaline. Stippled area indicates the 95% confidence limits of the response estimate.

a. Responses expressed as kPa ml^{-1}min.

b. Responses expressed as % change in resistance over baseline resistance.


a
3·0
2·0
ΔK
1·0
11
10
9
8
7
6
5
4
3
- log10 Concentration M


b
300
200
ΔK%
100
11
10
9
8
7
6
5
4
3
- log10 Concentration M

II.6.2.5 Infusion dose response curves for ISO

Isoprenaline in the perfusate at concentrations below 1 x 10^{-4}M decreased resistance. Concentrations of 1 x 10^{-4} and 1 x 10^{-3}M ISO caused increased resistance (see figure II.13). ISO dose response curves have slopes between 5.7 and 6.6. The ED50s were between 8.1 and 12.2 x 10^{-9}M. Addition of 5% human serum to the perfusate caused the following changes in the ISO dose response curves.

1. The ED50s were depressed slightly.

2. The maxima were significantly greater by 2.15 (ΔK) to 1.17 (ΔK%) times.

Records of responses to each of these drugs are presented in figure II.14.

To summarise, dose response curves to AD, NAD, and ISO are sigmoid. Adrenaline is 1.14 to 4.43 times more potent an alpha agonist than NAD. Isoprenaline decreased resistance at concentrations below 1 x 10^{-4}M. Perfusate containing 5% human serum enhanced the responses to AD and ISO but had little effect upon the values of the ED50s of these drugs.

II.6.2.6 Bolus dose response curves to AD, NAD, and ISO

The dose response curves for bolus administered AD, NAD, and ISO are presented in figures II.15 to II.17. Data from which the curves were prepared are tabulated in appendices A.5-A.8 (AD), A.11 and A.12 (NAD) and A.17-A.20 (ISO). Body contractions prevented determination of maximal responses by this method, consequently the curves are fitted by eye. Typical responses to boli of AD, NAD and ISO are presented in figure II.18. Table II.4 summarises the information from bolus dose response curves and is equivalent to table II.3 for infused drugs.

II.6.2.7 Bolus dose response curves for AD and NAD

Bolus dose response curves for AD are presented in figure II.15, and those for NAD in figure II.16. The curves for AD and NAD are very similar. Treshold doses for AD were slightly lower than for NAD. When perfused with 5% human serum in the saline, the responses to AD were smaller than with the basic saline (see section II.6.3.1).

II.6.2.8 Bolus dose response curves for ISO

Bolus dose response curves for ISO are presented in figure II.17. The curves from preparations perfused with the basic saline show

FIGURE II.13 Dose response curves constructed from peak responses to infusion of 1 x 10^{-11}M to 1 x 10^{-5}M isoprenaline. Data from these curves are summarised in table II.3. All responses were recorded while perfusing at caudal venous pressures of 1.33 kPa. Responses to 1 x 10^{-4}M and 1 x 10^{-3}M isoprenaline were omitted since they were increases in resistance. It is likely that these increases in resistance at very high isoprenaline concentrations reflected significant alpha constrictory adrenergic receptor stimulation by this predominantly beta agonist, (see appendices A.13-A.16 for the magnitude of resistance increases in response to high concentrations of isoprenaline). Stippled area indicates the 95% confidence limits of the response estimates.

a. Responses expressed as kPa $ml^{-1}min$. No serum in the perfusate.

b. Responses expressed as % change in resistance over baseline resistance. No serum added to perfusate.

c. Responses expressed as kPa $ml^{-1}min$. 5% human serum added to the perfusate.

d. Responses expressed as % changes in resistance over baseline resistance. 5% human serum added to the perfusate.

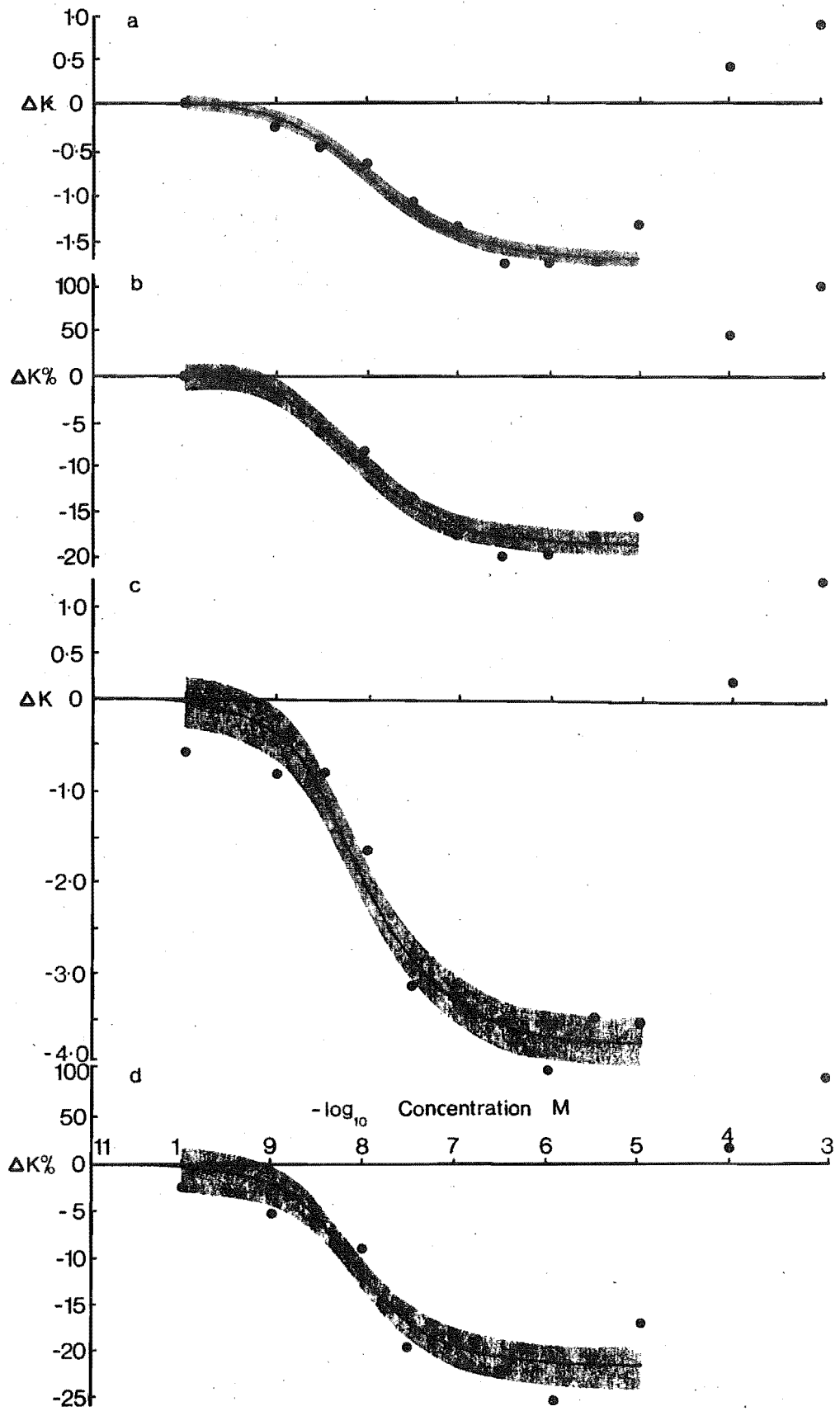

a
1·0
0·5
ΔK 0
-0·5
-1·0
-1·5
b
100
50
ΔK% 0
-5
-10
-15
-20
c
1·0
0·5
ΔK 0
-1·0
-2·0
-3·0
-4·0
d
100
50
ΔK% 0
-5
-10
-15
-20
-25
- log10 Concentration M
11
1
9
8
7
6
5
4
3

FIGURE II.14a Typical record of response to 5×10^{-7}M adrenaline. Note the decrease in caudal artery pressure after the initial peak. Preparation number ADB203. Tail weight = 272 g. Tail resistance change = +94.16%.

FIGURE II.14b Typical record of response to 1×10^{-4}M noradrenaline. There is no initial peak in caudal artery pressure as for adrenaline responses. Preparation number N113. Tail weight = 302 g. Tail resistance change = +191.75%.

FIGURE II.14c Typical record of response to 1×10^{-7}M isoprenaline. Note the slower development of the response compared to the constrictory responses above. Preparation number I202. Tail weight = 263 g. Tail resistance change = -21.27%.

LH lymph heart beats
CA caudal artery pressure, kPa
CV caudal vein pressure, kPa
OQ outflow, drops
O_2 venous oxygen tension

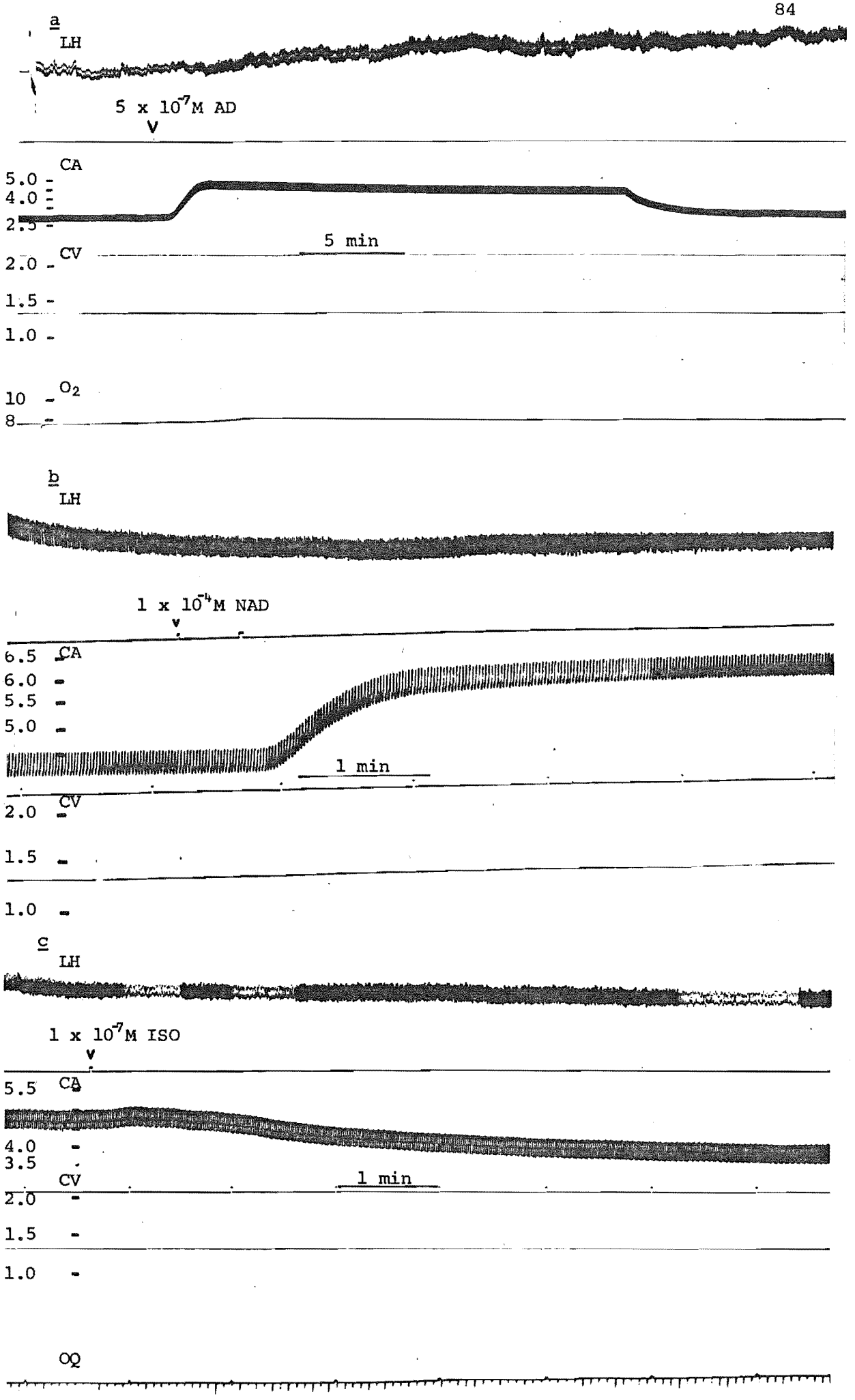

a
LH
5 x 10⁻⁷M AD
CA
5.0
4.0
2.5
5 min
CV
2.0
1.5
1.0
10
O₂
8
b
LH
1 x 10⁻⁴M NAD
6.5
CA
6.0
5.5
5.0
1 min
CV
2.0
1.5
1.0
c
LH
1 x 10⁻⁷M ISO
5.5
CA
4.0
3.5
CV
1 min
2.0
1.5
1.0
O₂

FIGURE II.15 Dose response curves constructed from peak responses to injection of 1 pmole to 100 nmoles of adrenaline. Data from these curves are summarised in table II.4. All the responses were recorded while perfusing at caudal venous pressures of 1.33 kPa. Curves were fitted by eye. Error bars = ± 1 S.E.M.

a. Responses expressed as kPa $ml^{-1}min$. No serum in perfusate.

b. Responses expressed as % change in resistance over baseline resistance. No serum in perfusate.

c. Responses expressed as kPa $ml^{-1}min$. 5% human serum added to the perfusate.

d. Responses expressed as % resistance change over baseline resistance. 5% human serum added to perfusate.

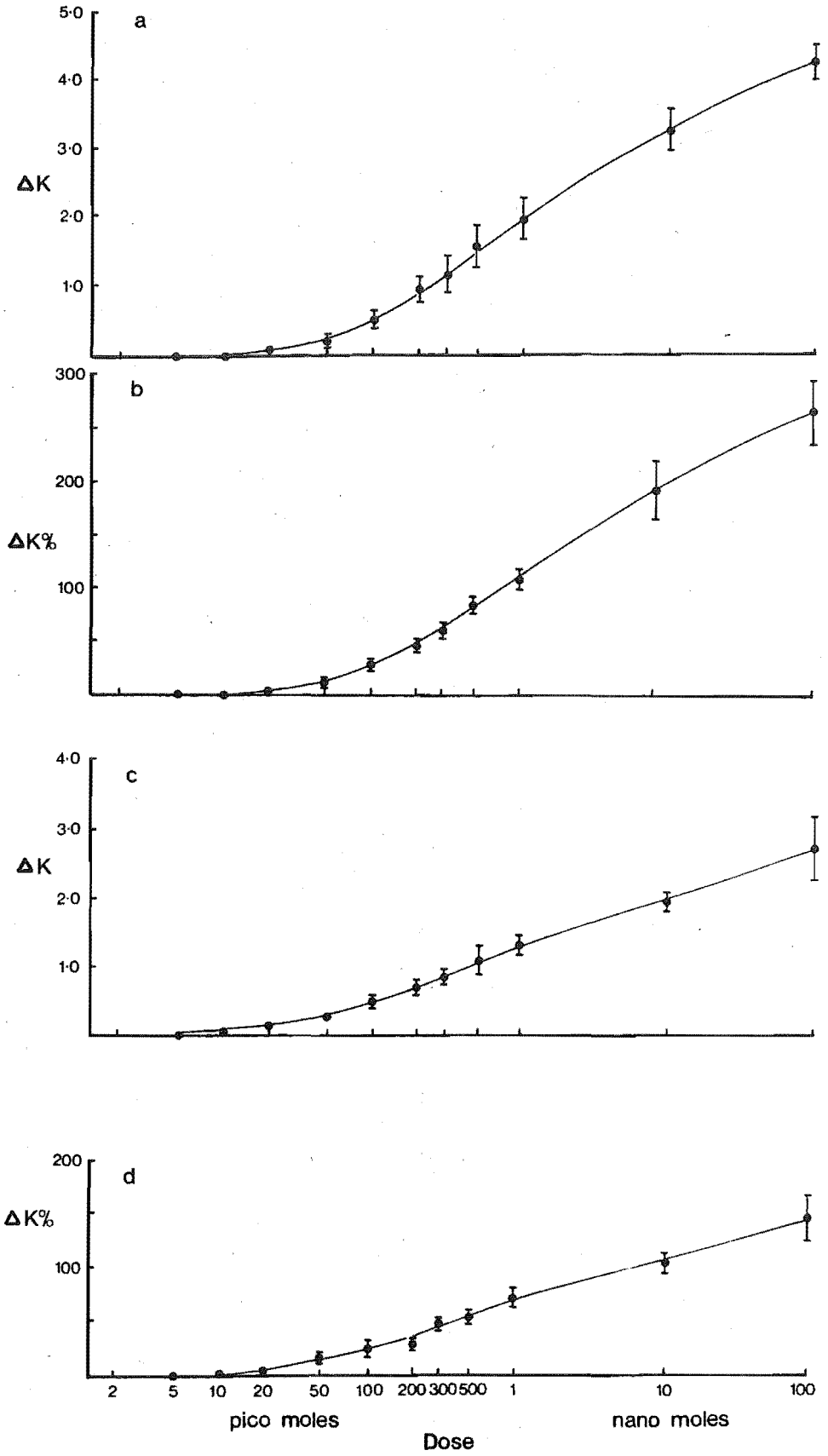

a
5·0
4·0
3·0
ΔK
2·0
1·0
b
300
200
ΔK%
100
c
4·0
3·0
ΔK
2·0
1·0
d
200
ΔK%
100
2
5
10
20
50
100
200
300
500
1
10
100
pico moles
nano moles
Dose

FIGURE II.16 Dose response curves constructed from peak responses to injection of 1 pmole to 100 nmoles noradrenaline. Data from these curves are summarised in table II.4. All responses were measured while perfusing at caudal venous pressures of 1.33 kPa and without serum in the perfusate. Curves were fitted by eye. Error bars are ± 1 S.E.M.

a. Responses expressed as kPa $ml^{-1}min$.

b. Responses expressed as % change in resistance over baseline resistance.

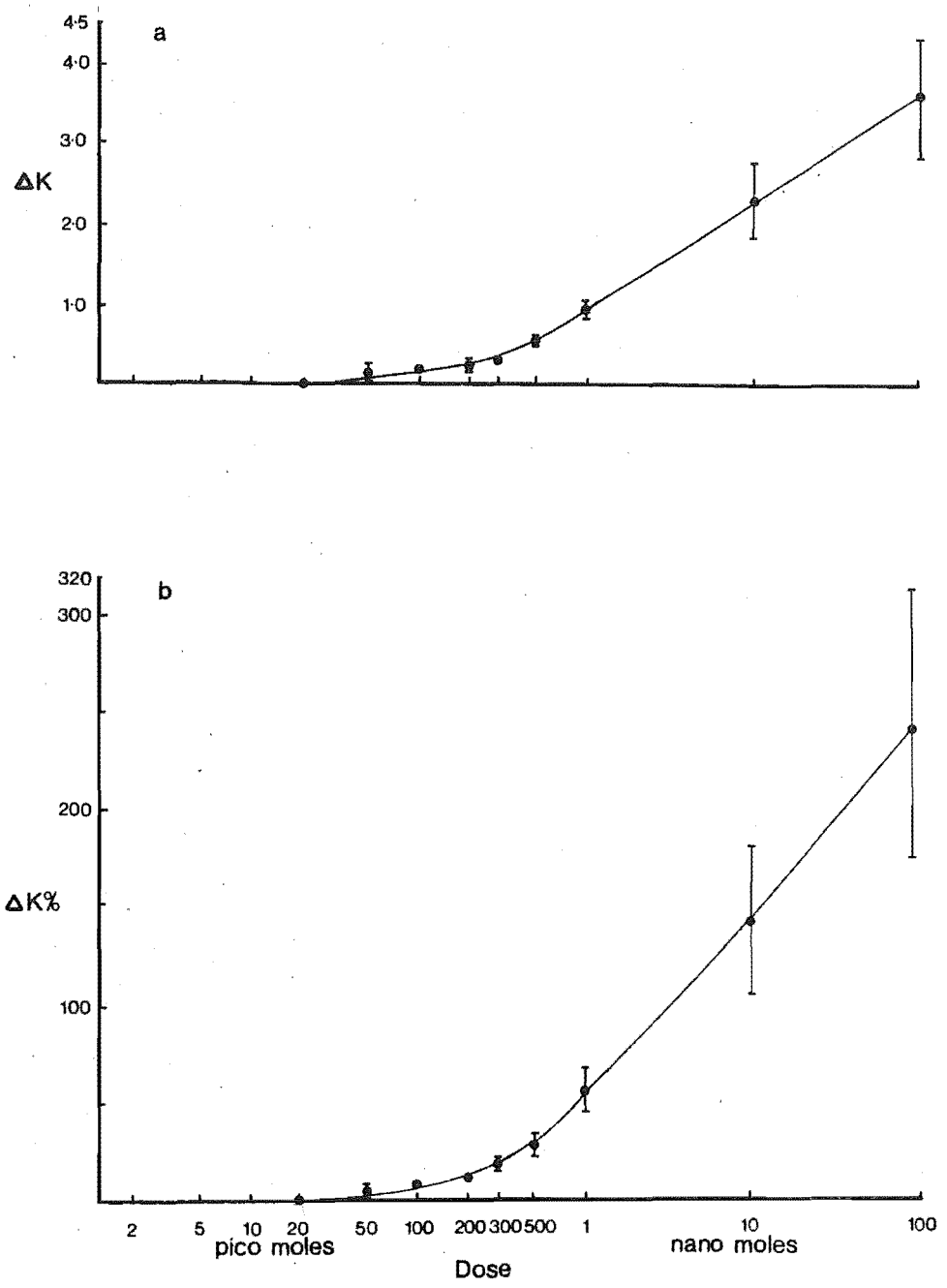

a
4·5
4·0
3·0
ΔK
2·0
1·0
b
320
300
200
ΔK%
100
2
5
10
20
50
100
200
300
500
1
10
100
pico moles
nano moles
Dose

FIGURE II.17 Dose response curves constructed from peak responses to injection of 1 pmole to 100 nmoles of isoprenaline. Data from these curves are summarised in table II.4. All responses were recorded while perfusing at caudal venous pressures of 1.33 kPa. Curves were fitted by eye. Error bars are ± 1 S.E.M. Curves in figure II.17b and II.17d show a trend toward a maximum response at the two highest doses. This may indicate some alpha adrenergic constrictory stimulation at these doses (see figure II.13a-d).

a. Responses expressed as kPa $ml^{-1}min$. No serum in perfusate.

b. Responses expressed as % change in resistance over basline resistance.

c. Responses expressed as kPa $ml^{-1}min$. 5% human serum added to the perfusate.

d. Responses expressed as % changes in resistance over baseline resistance. 5% human serum added to perfusate.

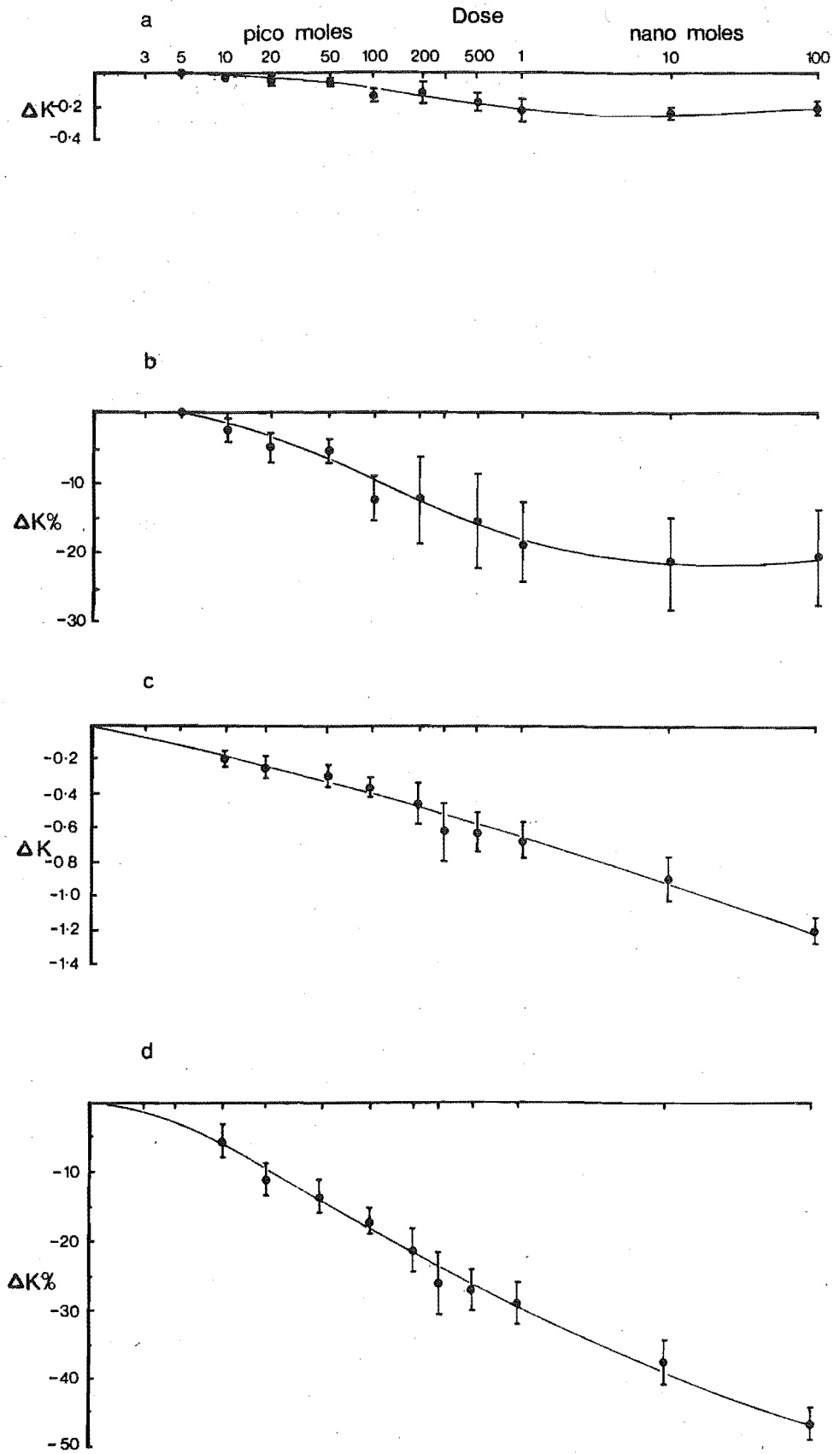

a
Dose
pico moles
nano moles
3 5 10 20 50 100 200 500 1 10 100
ΔK
-0·2
-0·4
b
-10
ΔK%
-20
-30
c
-0·2
-0·4
-0·6
ΔK
-0·8
-1·0
-1·2
-1·4
d
-10
-20
ΔK%
-30
-40
-50

FIGURE II.18a Typical response to injection of 10 nmoles adrenaline.
Preparation number A251. Tail weight = 186 g.
Resistance change = +62.66%.

FIGURE II.18b Typical response to injection of 10 nmoles noradrenaline.
Preparation number N158. Tail weight = 180 g.
Resistance change + 62.66%.

FIGURE II.18c Typical response to injection of 10 nmoles isoprenaline.
Note the first injection is a blank saline injection.
Preparation number I256. Tail weight = 282 g.
Resistance change = -28.0%.

LH lymph heart beat
CA caudal artery pressure, kPa
CV caudal vein pressure, kPa
OQ outflow, drops

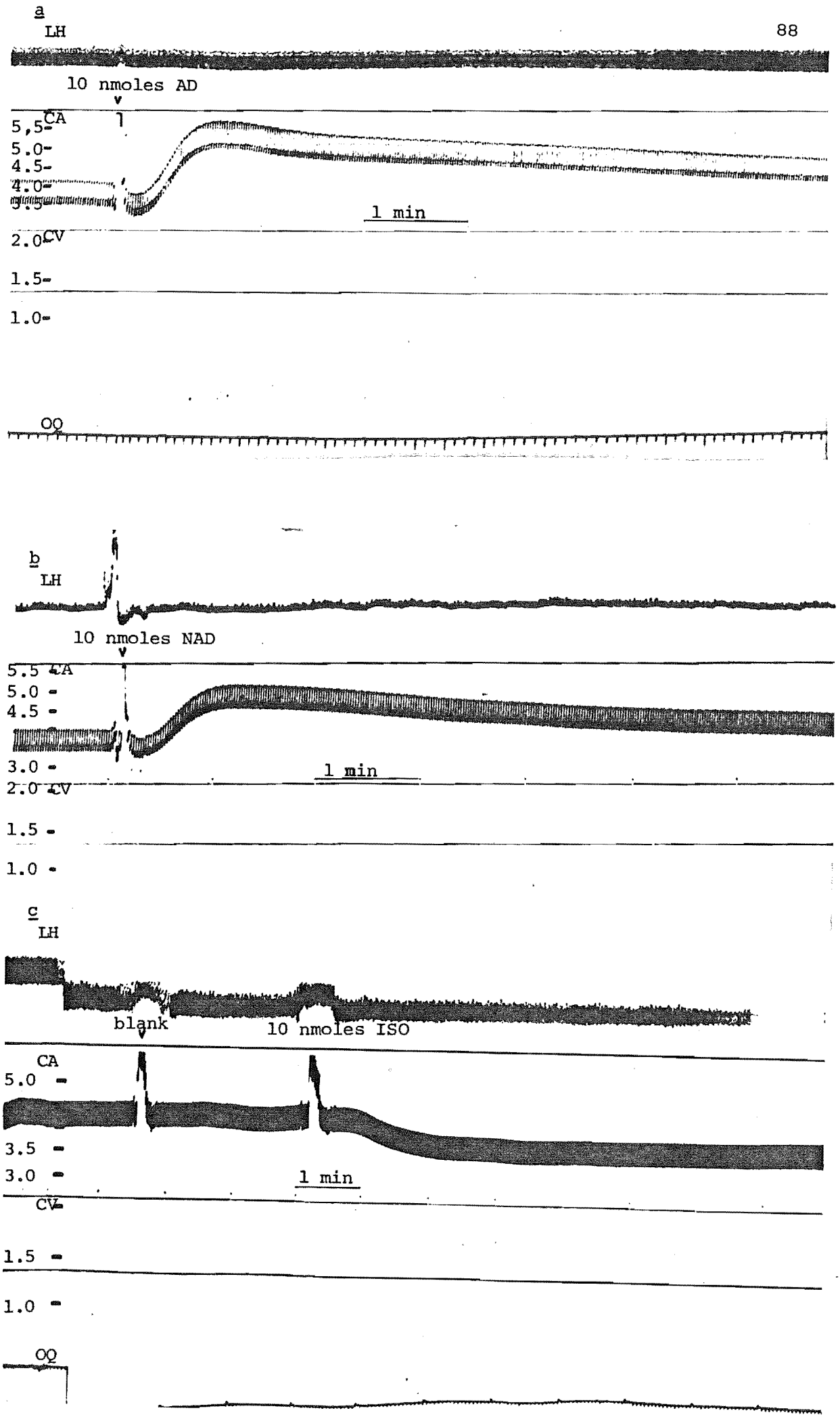

a
LH
10 nmoles AD
CA
5,5
5.0
4.5
4.0
3.5
1 min
2.0
CV
1.5
1.0
OQ
b
LH
10 nmoles NAD
5.5 CA
5.0
4.5
3.0
1 min
2.0 CV
1.5
1.0
c
LH
blank
10 nmoles ISO
CA
5.0
3.5
3.0
1 min
CV
1.5
1.0
OQ

Table II.4 Summary of results from bolus administration dose response curves.

no serum

		Adrenaline	Noradrenaline	Isoprenaline
Maximum ΔK (100nM) KPa ml^{-1} min		4.39 ± 0.47	3.57 ± 0.74	-0.226 ± 0.053
Maximum ΔK% (100nM)		267.4 ± 33.2	239.3 ± 65.5	-21.6 ± 6.7
Threshold dose		10-20 pmoles	20-50 pmoles	5-10 pmoles
Dose to produce half maximal response	ΔK	10.6 nmoles	2.1 nmoles	95 pmoles
	ΔK%	11.8 nmoles	9.1 nmoles	59 pmoles
Time to peak response s		74.1 ± 7.35	110.8 ± 9.54	231.6 ± 21.7

5% serum

		Adrenaline	Isoprenaline
Maximum ΔK (100nM)		+2.65 ± 0.45	-1.15 ± 0.067
Maximum ΔK% (100nM)		+147.5 ± 21.84	-46.4 ± 2.5
Threshold dose		5-10 pmoles	1-5 pmoles
Dose to produce half maximal response	ΔK	12.5 nmoles	29 pmoles
	ΔK%	11.8 nmoles	85 pmoles
Time to peak response s		66.2 ± 5.8	265.0 ± 23.6

Time to peak response for all doses are tabulated in appendices A.21 - A.22.

attentuation of the dilatory response at doses between 10 nmoles and 100 nmoles. Addition of 5% human serum to the perfusate increased responses to ISO by two (ΔK%) to five (ΔK) times.

II.6.3 Discussion of responses to sympathetomimetic drugs

II.6.3.1 The effects of serum on dose response curves for AD and ISO

Despite elevated baseline resistance, in the presence of serum, AD elicits greater absolute changes in resistance. We could envisage resistance vessels to have finite constriction limits. If serum produced a partial constriction, as indicated by increased baseline resistance, then there should be less potential for further vasoconstriction. The

fact that this is not the case demands a reappraisal of the action of serum on the vascular bed which enhances reactivity to AD. Serum could act to suppress the beta dilatory component of the AD response and allow AD to exhibit its full constrictory potential. However serum markedly enhanced the dilatory responses to ISO. Thus the development of beta responses appears to be in no way suppressed by serum, in fact quite the reverse.

It is well established that serum proteins can afford some protection to labile drugs, (see section II.4.4) and that transport of large molecules through capillary walls and interstitial spaces is faster in the presence of serum proteins. I propose that the increased reactivity of this preparation to sympathetomimetic drugs in the presence of serum is a result of two factors, namely protection, and enhanced mobility. Since we have no data available on the concentration of drugs in the immediate vicinity of the receptor sites, we assume that this concentration is the same as, or at least directly proportional to the concentration introduced. If catecholamines are associated with serum proteins then less free drug is available for catabolism and binding to non-reactive membranes, whilst more protein-drug complexes are being delivered to receptor sites more quickly.

Association of drugs such as AD and ISO with proteins and non-specific membranes in the tissue is assumed to be much weaker than receptor-drug binding (Gibaldi et al., 1978). Dissociation of drugs from protein molecules in the vicinity of receptors would result in the formation of relatively more stable receptor-drug complexes that would reduce the protein-drug complex concentration in the immediate area. The process is envisaged to be in equilibrium, and so drugs will be transported from non-reactive membranes, in association with mobile protein molecules that afford them some measure of protection, to receptors where the response will be enhanced by increased effective concentrations of the drugs. Stimulated Na^+ K^+ pump activity may also play some part at the muscle level (Smith and Rozengurt, 1978).

The fact that in the presence of serum larger absolute responses are developed indicating that without serum proteins the preparation fails to develop its full alpha or beta potential to exogenous catecholamines, despite supramaximal doses. This questions the concept that development of responses to active agents introduced via the perfusion line is limited by perfusion rather than diffusion across the capillary-cum-interstitial barrier. If responses to perfused drugs were delivery limited then the presence of serum should have little or no effect upon the maximum response observed.

The enhanced response to ISO in the presence of serum raises fewer questions. Elevated baseline resistance with serum should provide greater dilatory potential. Indeed maximum resistance changes are some two times greater when 5% human serum is added to the perfusate. Part of this may be due to the elevated baseline, but the possibility exists that part is also due to the protection and mobility effects of serum proteins.

Bolus administration of ISO in the presence of serum produced greater absolute and percentage resistance decreases. Adrenaline however was less potent when serum was added. One possible explanation for this could be that within the bolus, there is a higher proportion of drug to serum protein molecules. Given that the association between these molecules is weak, fewer drug molecules would be associated with proteins. Mixing of the bolus once administered with subsequent dilution was minimal. Reduced constrictory potential and greater dilatory potential caused by elevated baseline resistance could depress the responses of AD to a far greater extent and outweigh the enhancement effects of serum. In support of this notion it is noted that at low doses of AD, less than 50 pmoles, when the proportion of drug to serum protein is lower, the responses to AD in serum treated preparations is greater than those from preparations perfused with the basic saline.

Whatever the mechanism, serum increases the responsiveness of this preparation to AD and ISO. It should be noted that the ED50 estimated from ΔK or $\Delta K\%$ changes very little when serum is added. Only in the case of AD ΔK do the two ED50s fall outside their respective confidence limits, (see table II.3). In contrast to this ED50 estimates of different drugs are generally excluded from the limits of each other. The exception to this is provided by comparison of the ED50s of AD and NAD (ΔK) in the absence of serum, where the curves are very similar. A possible reason for this could be that the greater alpha adrenergic potency of AD is being masked by the simultaneous beta stimulation thereby reducing the responses fortuitously close to those elicited by NAD.

II.6.3.2 Comparison of responses to AD and NAD

Adrenaline is a more potent alpha agonist than noradrenaline. Comparison of ED50s from the infusion data show AD to be 1.14 to 4.31 more potent than NAD for ΔK and $\Delta K\%$ estimates respectively. The use of ED50 comparisons is preferable when only one receptor is being investigated. However when two receptors are present the situation

requires closer examination.

The data points for the NAD curve show a rather marked deviation from sigmoidicity at higher concentrations (see figure II.12). At 1×10^{-4}M the response is above the estimated assymptotic maximum, while at 1×10^{-3}M it is well below the curve. This behaviour suggests that an insignificant proportion of beta receptors are stimulated by NAD at concentrations below 1×10^{-3}M and the response is almost entirely due to alpha stimulation alone. However at the highest concentration, 1×10^{-3}M, some beta stimulation is evident. When this is superimposed upon maximal alpha stimulation the result is a downturn in the dose response curve. Adrenaline on the other hand elicits significant beta activity at much lower doses, and certainly at 5×10^{-4}M (see section II.7) causing the dose response curve to be depressed along its entire length. The downturn at high concentrations of AD is not so marked. This indicates that AD is a considerably more potent beta agonist than NAD and the beta receptors must be of the beta two variety (Ahlquist, 1948; Furchgott, 1967; Arnold, 1972; Wood, 1976).

The greater beta activity of AD seems likely to be the cause of the lower maximum constriction when compared to NAD, (2.64 kPa ml^{-1}min and 2.941 kPa ml^{-1}min, AD and NAD respectively). Despite the greater response depression by beta stimulation, the AD ED50 is lower than that of NAD. If the responses at 1×10^{-5}M are compared, then the absolute responses are very similar, but the percentage change in resistance over baseline resistance is greater for AD by ~ 1.38 times.

Comparisons of responses to equimolar bolus doses of AD and NAD (1 nmole), in the absence of serum show that AD is ~ 1.38 times more potent a constrictor than NAD, which is in accord with the results from the curves prepared from infusion of the drugs.

II.6.3.3 Comparison of the two methods of drug administration

To compare the two methods of drug administration, the half maximal response was calculated from the curves when the drugs were administered by the perfusion line. The half maximal response was then located on the ordinate of the appropriate bolus dose response curve and the dose required to produce this response read off.

Since the peak response times are less than, (AD and NAD), or of the same order as (ISO) the circulation time through the tail (see section II.5.4) all of the drug contained in the bolus is assumed to be within the preparation vascular space. The concentration of drug in the vascular space at the peak of the response during perfusion administration

is assumed to be the concentration made up. These assumptions are crude but in the absence of detailed knowledge of the exchange kinetics of the vascular space and other spaces within the preparation they must serve as a first order approximation to the real situation. If we assume that the vascular space is about three mls per 100 g of tissue, (see Thorson, 1961 and section II.7.2) and the average tail weight to be around 200 g, then the amount of drug present in the tail is 6 x 10^{-3} ℓ times the concentration in moles per litre for perfusion administration. The results from these estimates are presented in table II.5.

Table II.5 Comparison of ED50 for AD, NAD, and ISO when administered as a bolus and by infusion at constant concentration.

	Adrenaline	Noradrenaline	Isoprenaline
Mean ED50 perfusion line	24 nmoles	58 nmoles	57 pmoles
Mean ED50 bolus	11.6 nmoles	5.6 nmoles	67 pmoles

Responses to the same amount of drug are surprisingly similar for the two methods of administration except in the case of NAD. NAD given as a bolus appears to be about ten times more potent than by perfusion. If however the ED50 estimate from the ΔK curve alone is used then the perfusion line ED50 is about 3.5 nmoles. Because of the low slope of the NAD $\Delta K\%$ curve, the ED50 estimate would be expected to be less accurate. Thus the difference could be due to this, rather than a real difference in action of the drug introduced by different means. Within the limitations of this comparison there appears to be no difference in response to the same amount of drug given as either a bolus or via the perfusion line.

II.6.4 Sympathetomimetic antagonists

II.6.4.1 Introduction

To further elucidate the adrenergic system in the eel tail vasculature three adrenergic blocking agents were employed. Phentolamine (Phent) and propranolol (Prop) were the principal alpha and beta blockers respectively. Dichloroisoproterenol (DCI) was occasionally used for beta blockade. Each of these antagonists acts in a competitive manner (McDevitt, 1977). These studies also revealed that the blockers had effects on tail vascular resistance in the absence of any agonist.

II.6.4.2 Phentolamine blockade of the response to AD

Eight preparations, each of which had previously shown a vasodilatory response to ISO were given various combinations of AD and Phent. The responses to Phent alone were determined in three preparations at least two hours after the last drug administration. Three low doses of AD (50 pmoles, 100 pmoles, 200 pmoles) were given to the preparation. These control responses were compared to the responses to the same dose of AD when Phent was present. After the blockade experiments the control doses of AD were again administered and the response was always found to be diminished by 30% to 50% compared to the initial controls. This could be the result of either incomplete washout of Phent or the loss of sensitivity of the receptors to AD. Loss of sensitivity to AD is not apparent in the absence of blockers especially at the low doses that were chosen to specifically avoid the problem. The other reasons for the choice of low doses of AD is that in order to obtain AD to Phent concentration ratios of up to 1 : 1000 at even moderate doses of AD the amounts of Phent necessary caused muscular contractions. Muscular contractions were most undesirable since they produced wildly fluctuating pressure records and unreadable lymph heart records.

After the control responses were recorded, the amount of Phent in the 0.1 ml bolus was raised to give ratios of AD to Phent of between 1 : 1 and 1 : 1000. The results from these experiments are presented graphically in figure II.19 and tabulated in appendix A.23. The increase in resistance caused by AD was diminished by the presence of Phent. At each of the three doses of AD used the null response occurred at a concentration ratio of AD to Phent of 1 : 35 to 1 : 45, suggesting that effective alpha blockade occurred at this level. At higher agonist to antagonist concentration ratios the response changed from vasoconstriction to vasodilation. The control doses of Phent alone caused dilation. The magnitude of these responses was however too small to account for the dilatory response to AD plus Phent at concentration ratios above 1 : 45 (see appendix A.24). These results demonstrate AD mediated dilation during alpha blockade.

To further investigate the nature of the dilation caused by AD plus Phent, the beta blocker DCI was used. When AD plus Phent plus DCI were given simultaneously at concentrations of 200 pmoles, 40 nmoles, 40 nmoles respectively, (dose ratio of 1 : 200 : 200) to two preparations, the dilatory response was abolished or markedly diminished. By further increasing the dose of DCI the response was always abolished,

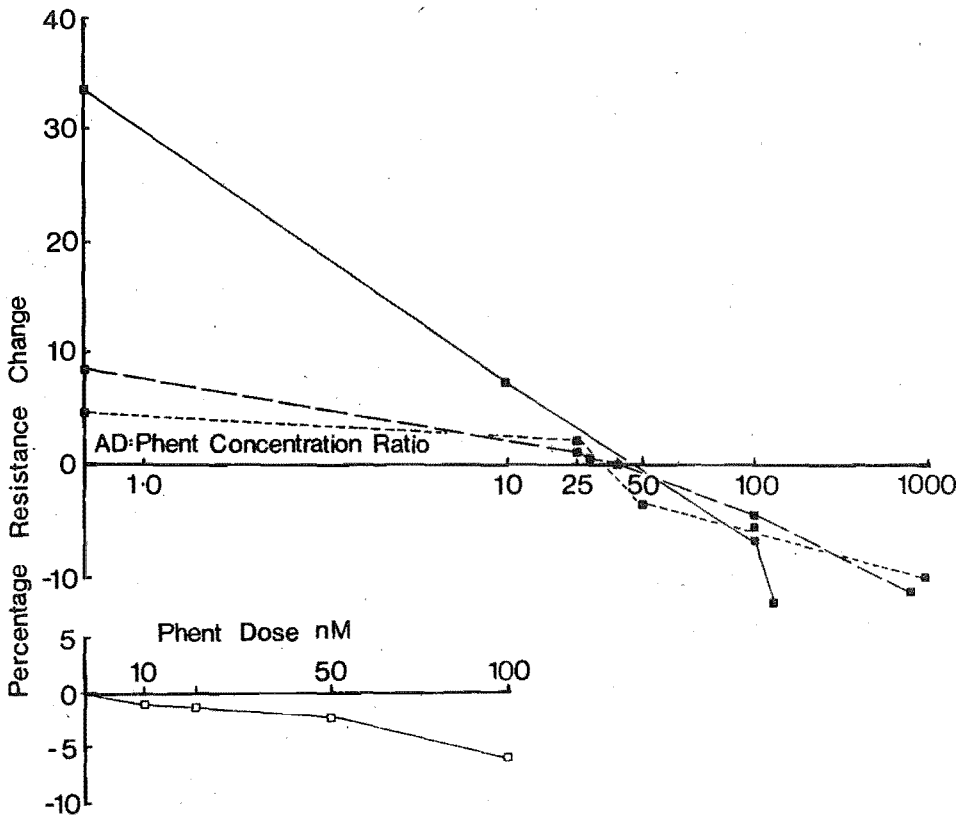
FIGURE II.19 Effects of increasing concentration ratios of phentolamine on the % resistance responses to 50, 100, and 200 pmoles adrenaline. Responses to control doses of phentolamine are presented on the same graph. The constrictory responses to adrenaline at these doses were reversed to dilation at a concentration ratio of about 1 : 40 adrenaline : phentolamine. Dilatory responses to adrenaline in the presence of the alpha blocker phentolamine may be partly due to the antagonist alone.

—■— 200 pmoles

—■- 100 pmoles

···■··50 pmoles

—□— Phentolamine


40
30
20
10
0
-10
Percentage Resistance Change
AD:Phent Concentration Ratio
1·0
10
25
50
100
1000
Phent Dose nM
5
0
-5
-10
10
50
100

(1: 200 : 300). The administration of both and alpha and beta blocker to the preparation represents a terminal experiment and was performed at the end of a preparation's useful life. Because of the contrived nature of the conditions and the fact that the preparation had been exposed to AD, ISO and Phent before DCI was administered, these results must be accepted with caution. They do however indicate that the dilatory response to AD during alpha blockade was the result of beta stimulation.

In two preparations AD and Phent were administered simultaneously via the perfusion line. When 1 x 10^{-8}M AD was given with Phent at a concentration of 1 x 10^{-7}M (1 : 10) there was a slight reduction in the response from +20.4% to +18.8%. When a concentration of 1 x 10^{-6}M Phent was used (1 : 100), a dilation of -10.2% was observed. When a concentration of 1 x 10^{-5}M Phent was used (1 : 1000), gross muscular contractions ensued making measurements of resistance very difficult. No rise in resistance above that produced by 1 x 10^{-8}M AD was seen during these experiments.

Control doses of 1 x 10^{-7}M and 1 x 10^{-6}M Phent had no detectable effect on resistance. At 1 x 10^{-5}M Phent there was a small decrease of -4.08 ± 2.27% (n = 4) (see appendix A.24).

One preparation was perfused with 1 x 10^{-6}M, 1 x 10^{-5}M, and 1 x 10^{-4}M Phent sequentially. After ten minutes of perfusion with each concentration, 300 pmole doses of AD were administered. The responses to AD were decreased to about 40% of the control response but the effects of increased Phent concentrations were not apparent (see appendix A.25).

Two features emerge from the use of AD plus Phent. The first is that Phent did not abolish the resting tone found in preparations perfused with plain ringer or ringer that had 5% human serum added to it. The basal tone is not solely the result of any residual alpha adrenergic stimulation. Control doses of Phent elicited small dilatory responses. This could be due to the blockade of the small part of the resting tone that is due to alpha stimulation (incomplete washout of alpha agonists), or to some non-specific stimulation of beta receptors by the alpha antagonist Phent. Since the responses to Phent were always recorded after the administration of AD and Phent is a highly selective alpha antagonist the former explanation seems the most likely.

The second point is that AD mediated vasodilation in the presence of an alpha blocker was difficult to demonstrate. The most convincing evidence came from administration of AD and Phent together in a single

bolus. Although this evidence is clear, it is still complicated by the effects of the antagonist alone and cannot be rated as unequivocal. An alternative method to demonstrate AD mediated beta stimulation is to apply a selective beta blocking agent during the response of the preparation to AD and watch for potentiation of the constrictory response. This approach will be considered separately, (section II.6.4.4)

II.6.4.3 Beta blockade of ISO mediated vasodilation

The effect of propranolol (Prop) and dichloroisoproterenol (DCI) on the dilatory responses to ISO of eight preparations were recorded. Responses to control doses and concentrations of Prop and DCI are tabulated in appendix A.29. Administration of a single bolus containing 200 pmoles ISO plus Prop at concentration ratios of 1 : 1 to 1 : 20 reduced the magnitude of the dilatory responses by 40 - 70% (appendix A.26). The residual dilatory responses at high concentrations of Prop could have been due to Prop alone (see appendix A.29). The rapid return to baseline resistance after dilation in response to Prop alone (< 5 minutes) led to injection of Prop at similar concentration ratios as the single boli experiments ten minutes before injections of ISO at doses of 200 pmoles (appendix A.26). The magnitude of the dilatory responses to ISO in these experiments was reduced by 90-97%. ISO plus DCI in single boli at concentration ratios of 1 : 100 to 1 : 1000 showed reduced dilatory responses compared to control responses (appendix A.27). The reduction of the magnitude of the dilatory responses was smaller than with propranolol (~ 65%). Control doses of DCI also resulted in dilatory responses which were reversed to constriction at higher doses and concentrations (> 1 μmole, > 1×10^{-4}M). Because of the reversal found in responses to control doses of DCI, indicating lack of receptor specificity, the use of DCI was not favoured. Prop, although having greater inherent dilatory activity was a more potent beta antagonist, consequently it was used in most experiments requiring a beta antagonist.

When the tail was perfused with 1×10^{-7}M ISO plus 1×10^{-6}M DCI, (concentration ratio, 1 : 10) the dilatory response was about one tenth of the response to 1×10^{-7}M ISO alone. On increasing the concentration ratio to 1 : 100, there was no detectable response. At a ratio of 1 : 1000 an increase in resistance of about +35% over the baseline was observed. A similar trend was noted when 1×10^{-6}M ISO was used and the concentration of DCI raised progressively (see appendix A.28). The constriction in the presence of high concentrations of DCI could be due either to alpha stimulation by ISO in the absence of available beta

receptors, or to a non-specific stimulation of alpha receptors by DCI. The former explanation is likely since at the concentrations of ISO used the beta response is at or near its maximum in unblocked preparations. At higher ISO concentrations constriction was found which may well be the result of alpha stimulation. If beta sites were blocked by DCI then the effective ISO activity may be sufficient to initiate constriction. In the presence of the alpha antagonist Phent, ISO dilatory responses were potentiated (see appendix A.30).

The effects of the beta antagonists Prop and DCI on the dilatory responses of the eel tail to ISO was to diminish them. Prop was a more potent beta antagonist than DCI and reduced the magnitude of responses to ISO by 70-97% at concentration ratios of 1 : 1 to 1 : 10.

II.6.4.4 Propanolol and the response to AD

Prop was found to be a potent beta antagonist in this preparation. The response to AD is assumed to be the sum of the opposed alpha (constrictory) and beta (dilatory) responses. Blockade of the alpha component failed to clearly reveal substantial beta activity. The alternative was to block the beta response and from the above hypothesis we could expect to see an enhanced vasoconstriction in response to AD. Such an enhanced constriction would be against the inherent dilatory activity of Prop on its own, and thus if observed could not be explained by the presence of the antagonist.

Nine preparations were used to study the effects of Prop on the response of the tail to AD. Prop and AD were administered as a single bolus or as separate boli. In the case of separate boli Prop preceding AD by 5 minutes, which was the time taken for the recovery of the preparation from Prop alone. These methods always produced a smaller rather than larger constrictory response to AD (see appendix A.31). The dose of AD was varied between 10 pmoles and 1 nmole at concentration ratios of AD to Prop of 1 : 5 to 1 : 100 yet the nature of the response remained the same, namely a depression of the constriction caused by AD (see figure II.20). The only indication that Prop was present was the decreased resistance caused by Prop, as observed in Prop controls, superimposed upon the smaller increases caused by AD, (see figure II.21). When DCI was used instead of Prop similar results were obtained.

These results are at variance with the proposed hypothesis for the action of AD and need to be carefully examined. The time to reach peak responses to control doses of both AD and Prop were of the order of 45-70 s. The time to peak responses to ISO were around 200-250 s. This

FIGURE II.20 Effects of propranolol, a beta adrenergic antagonist, on the responses to 5 pmoles to 1 nmole adrenaline. Propranolol diminished the constrictory responses to adrenaline when compared to the control responses from the same preparations. If propranolol blocked only the beta dilatory adrenergic receptors then an enhanced constrictory response to adrenaline might be expected. That this was not the case suggests that propranolol has significant alpha blocking potential in the eel tail (for discussion see text). Propranolol was administered at 100 times the concentration of adrenaline simultaneously with adrenaline (see appendix A.31).

—■—Control responses

—□—AD plus Prop

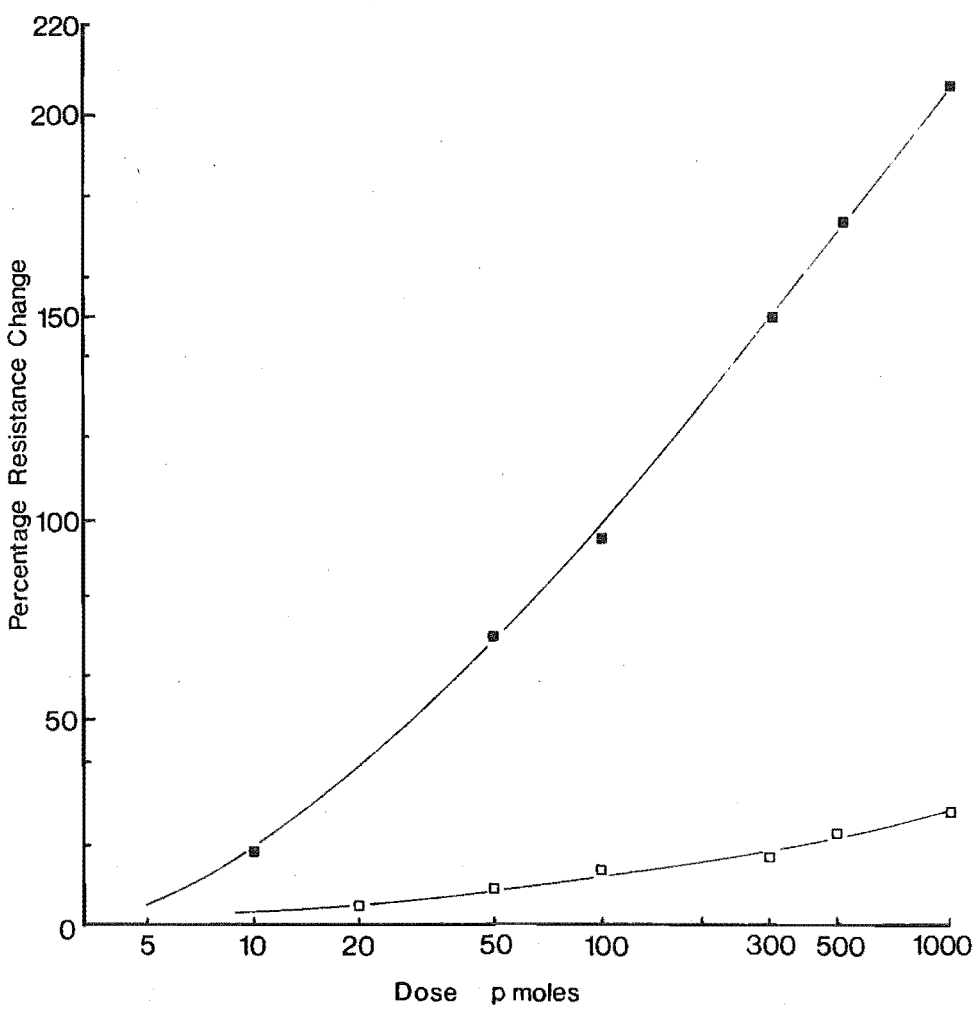
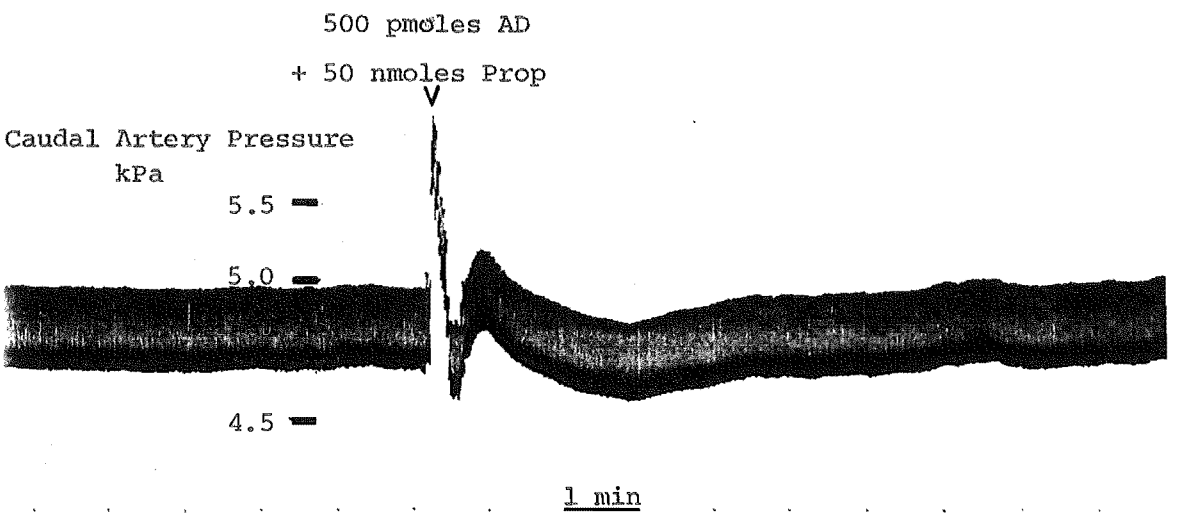


FIGURE II.21 Typical record of response to a single bolus containing adrenaline plus propranolol at doses of 500 pmoles and 50 nmoles respectively. The reasons for depression of the constrictory response to adrenaline by propranolol are discussed in the text.


500 pmoles AD
+ 50 nmoles Prop
Caudal Artery Pressure
kPa
5.5
5.0
4.5
1 min

raises the question of where the dilatory response to Prop originates. If Prop causes some beta stimulation then the qualitative features of the response (for example the shape of the response trace) and the time course could be expected to fit with these features of the response to ISO stimulated dilatory responses. The time course and the shape are markedly different from those of the responses to ISO. An alternative is that Prop could be blocking the alpha response causing the observed decrease in resistance. The time course and the shape of the response to Prop is very similar to that of AD, but in the opposite direction. Thus if we assume Prop to have some alpha antagonistic activity, then to demonstrate greater alpha activity during beta blockade with these drugs then we could first occupy as many of the alpha sites with AD before the introduction of Prop.

To this end five preparations were perfused with 5×10^{-7}M AD for 10 to 20 minutes. After this period the perfusate was immediately changed to the following solutions, each being perfused for 10 minutes.

5×10^{-7}M AD plus 5×10^{-7}M Prop (1 : 1)
" " 2.5×10^{-6}M Prop (1 : 5)
" " 5×10^{-6}M Prop (1 : 10)
5×10^{-7}M AD

The results of these experiments are presented in tables II.6, II.7 and in figure II.22. The elevated resistance level caused by AD was raised even higher in the presence of 1 : 1 and 1 : 5 AD to Prop. However when 1 : 10 AD to Prop was infused, the resistance fell to a level near that of the response to 1 : 1 AD to Prop. When Prop was removed from the perfusate and only AD was perfused through the preparation the greatest resistance increase was observed and was significantly greater than the control resistance increase ($P < 0.01$, Student's t-test). This response peaked at around 330 s after Prop was removed.

The peak of the response to 5×10^{-7}M AD occurred at ~ 145 s. For ISO at the same concentration the peak response occurred at ~ 300 s. The beta response is slower to develop than the alpha response. Thus for the first 300 s of perfusion with Prop free 5×10^{-7}M AD after solutions containing AD plus Prop, any Prop occupying alpha or beta sites was being washed out since Prop was no longer being replaced from the incoming perfusate. As Prop left the receptors, alpha or beta, it was replaced by AD. However, because the constrictory response was more rapidly developed than the dilatory response there was a rise in resistance until the beta response developed. This explanation of the

Table II.6 The effects of the beta antagonist propranolol on the resistance response of the perfused eel tail to adrenaline (n = 5).

	AD control	5×10^{-7}M	1 : 1		1 : 5		1 : 10		AD control
	Peak	Plateau	AD	Prop	AD	Prop	AD	Prop	5×10^{-7}
ΔK kPaml^{-} min	2.59 ± 0.51	2.14 ± 0.41	2.71 ± 0.47		2.91 ± 0.33		2.61 ± 0.29		3.61 ± 0.45
ΔK%	126.32	104.63	134.41		145.34		130.64		178.22
Time (mins)	1.5	10	10		10		10		5.5

Table II.7 The resistance response of the perfused eel tail to propranolol at the concentrations used in table II.6 (n = 4).

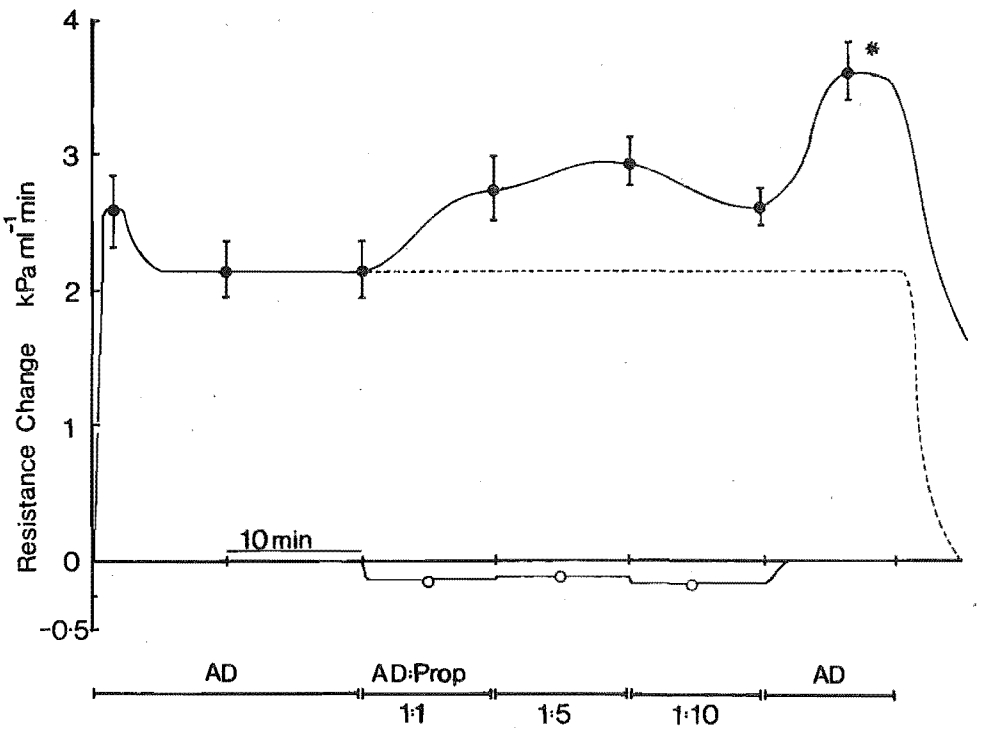
Propranolol concentration	5×10^{-7}	2.5×10^{-6}	5×10^{-6}
ΔK kPa ml^{-} min	-0.152 ± 0.037	-0.144 ± 0.059	-0.207 ± 0.034
ΔK%	-7.73 ± 2.39	-5.44 ± 1.51	-9.69 ± 0.303

FIGURE II.22 Mean resistance responses to infusion of 5×10^{-7}M adrenaline followed by 5×10^{-7}M adrenaline plus propranolol at concentrations of 5×10^{-7}M, 2.5×10^{-6}M and 5×10^{-6}M followed by 5×10^{-7}M adrenaline again. Responses to propranolol at the concentrations used are displayed on the same figure. Error bars are ± 1 S.E.M. (n = 5). Student's t-test between mean responses to each drug solution combination and the initial control response to adrenaline alone showed that only during infusion of adrenaline alone (*) after infusion of adrenaline with propranolol was there a significant increase in the response ($P < 0.01$). See text for discussion.

—●— Resistance responses throughout experimental series of drugs

····· Represents the expected response in the absence of propranolol

—○— Resistance responses to control doses of propranolol


4
3
2
1
0
-0·5
Resistance Change kPa ml^{-1} min
10 min
*
AD
AD:Prop
1:1
1:5
1:10
AD

nature of the responses represented by figure II.22 seems plausable and shows that there is a large potential for AD mediated vasodilation in the eel tail of about 40-50% of the alpha response to AD.

Experiments involving the use of adrenergic antagonists support the results from agonist administration. Increased resistance in response to AD was blocked by the alpha antagonist Phent. Decreased resistance in response to ISO was blocked by Prop and DCI. Responses to AD were the sum of increases in resistance caused by alpha stimulation plus decreases in resistance caused by beta stimulation. The beta response to AD was about 40-50% of the magnitude of the alpha response to AD. Phentolamine appears to be a very selective and potent alpha antagonist in this preparation. Both Prop and DCI exhibited partial alpha antagonism as well as the predominant beta antagonism. Propranolol was a more potent beta antagonist than DCI in this preparation.

II.6.5 Effects of AD, NAD and ISO upon venous oxygen tension of the isolated saline perfused eel tail

II.6.5.1 Introduction

In the absence of respiratory pigment, the oxygen carrying capacity of vertebrate blood is reduced by at least 25 times, (Steen, 1971). A viable isolated tissue preparation is assumed to have a low 'basal' metabolic rate. Fish tail muscle often functions anaerobically, (Black, Chin, Forbes and Hanslip, 1959). Thus the oxygen requirements of this preparation are probably very low.

The metabolic cost of maintaining smooth muscle contractions in mammals is lower than that of maintaining skeletal muscle tension (Gluck and Paul, 1977). Vascular smooth muscle relaxes in response to lowered oxygen tension (Johnson, 1977), but will still maintain contractions in solutions containing more than 0.05 ppm (Pittman and Duling, 1973). Adrenaline mediated constriction depresses oxygen extraction of perfused tissues in a dose dependent manner (Pawlik, Shepherd and Jacobson, 1975). Dilation causes increased oxygen extraction in perfused vascular beds. Both of these changes are diffusion limited, rather than perfusion limited. Although perfusate oxygen tension may have significant effects upon the reactivity of the vascular bed, the results presented here clearly indicate adequate oxygenation of the perfused eel tail under all experimental conditions.

The perfusion medium was bubbled vigorously with 95% O_2/5% CO_2. This not only ensured a high input oxygen concentration but also a pH of

around 7.2. Increased osmolarity and decreased oxygen concentration and pH cause vascular dilation in mammals (Johnson, 1977). When studying changes in vascular tone these local ionic and 'metabolic' effects must be considered.

II.6.5.2 Results

Venous oxygen tension was measured as described in section II.3.3 in 12 preparations. The oxygen meter was zeroed in a 5% sodium dithionate solution and calibrated in air, and against air saturated distilled water before and after each preparation and found to be stable during the recording period. Drug solutions were always bubbled with 95% O_2/5% CO_2 for two to three minutes before the start of infusion and throughout infusion. Mean initial and final venous oxygen tensions were 11.21 ± 0.51 ppm and 11.41 ± 0.82 ppm (n = 12) respectively and were above the air saturated oxygen concentration of the ringer, (10.2 ppm at 10°C). Oxygen concentration in outflowing ringer was greater than 28 ppm when arterial and venous cannulae were connected by a short length of tubing, thereby excluding the tail from the circuit.

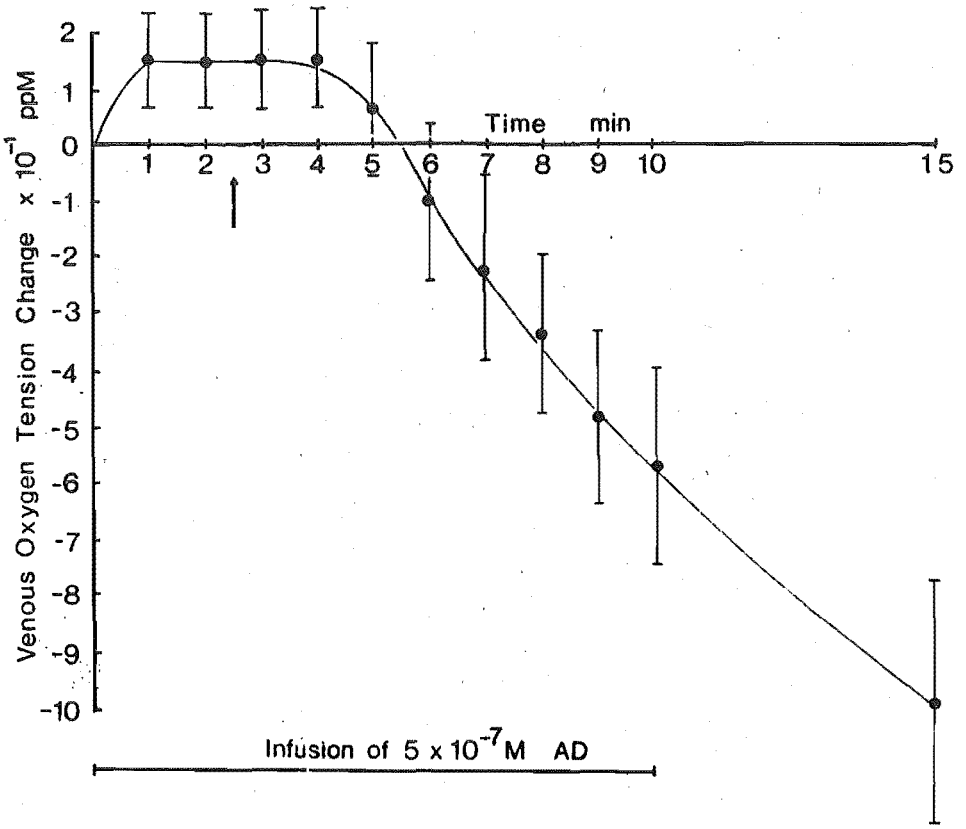
Infusion of NAD and AD and bolus administration and infusion of ISO reduced venous oxygen tension slightly ten minutes after drug introduction. The greatest recorded change in oxygen concentration was a fall of 1.4 ppm after 15 minutes of infusion of 5 x 10^{-7}M AD. Bolus administration of 20, 50, 200 and 500 pmoles of AD into one preparation produced small increases in venous oxygen tension after eight to ten minutes. The largest change was +0.8 ppm, 11 minutes after injection of 200 pmoles of AD.

In seven preparations venous oxygen tension was monitored during ten minutes of infusion of 5 x 10^{-7}M AD. The mean changes in oxygen tension are presented in figure II.23. All falls in venous oxygen tension showed a lag of the start of the decline of three to four minutes after the start of infusion. Four preparations were perfused with this concentration of AD for more than ten minutes. These preparations showed that oxygen tension reached a minimum within 15 to 20 minutes, and often started to rise slowly toward preinfusion levels thereafter.

II.6.5.3 Discussion

Eel skin has long been recognised as a potential respiratory surface and has been purported to provide up to 60% of the total oxygen requirements of the animal (Krogh, 1904). Kirsch and Nonnote (1977) have shown that little benefit to other than skin tissue results from

FIGURE II.23 Changes in venous oxygen tension (ppM) during each minute of infusion of 5×10^{-7}M adrenaline. Mean initial oxygen tension = 12.25 ± 0.66 ppM (n = 6). Error bars are ± 1 S.E.M. Arrow indicates peak resistance increase time. At no time were any of the preparations under hypoxic conditions.


Venous Oxygen Tension Change x 10^{-1} ppM
2
1
0
-1
-2
-3
-4
-5
-6
-7
-8
-9
-10
Time min
1
2
3
4
5
6
7
8
9
10
15
Infusion of 5×10^{-7}M AD

cutaneous respiration. The point to note however is that eel skin is permeable to oxygen. The perfusate loses about 60% of its oxygen content in its course through the tail. Part of this could be due to metabolism. However some may be lost through the skin to the bathing medium. A concentration gradient from the tissue to the bath persists during all experimental conditions. Thus it is not possible with these data to discuss the oxygen requirements of the preparation. What is clear is that at no time is the preparation under hypoxic conditions.

Adrenaline mediated elevated vascular resistance caused a fall in venous oxygen tension. This qualitatively reflects increased oxygen extraction by the preparation. Since flow is constant, oxygen delivery is constant and the additional loss of oxygen must be assigned to either increased metabolism or to increased transcutaneous loss. Because of the small amount of smooth muscle involved in resistance adjustments and its low oxygen demand it seems unlikely that active vasoconstriction caused the measured venous oxygen tension falls. Thus adrenaline infusion seems to have caused more perfusate oxygen to be lost either to the bath or to the tissue in general. Increased tissue oxygen is almost certainly not used since the muscles are metabolically quiescent and the tissue is already saturated with oxygen. An alternative explanation is that decreased venous oxygen tension is the result of additional loss through the skin.

Fish red muscle, which is used during continuous swimming (Hudson, 1973) is located just beneath the skin (Hulbert and Moon, 1978). Should the red muscles receive more perfusate during AD vasoconstriction then their anatomical position would indeed allow more oxygen to be lost to the bath. Adrenaline produces an increase in tail resistance, however the response is biphasic: an initial increase followed by a partial decrease (see figure II.14a). The beta dilatory activity of AD may be located within the red muscles which stand to benefit most from increased blood supply during swimming. The alpha constrictory component of the response to AD may direct blood away from the white muscles to the red muscles. That ISO also caused decreased venous oxygen tension lends some support to this notion.

II.6.6 Discussion of the adrenergic control of the eel tail vascular bed

II.6.6.1 The eel tail vascular bed and other teleost systemic vascular beds

Experimental design is especially important in pharmacological experiments where accuracy is often severly limited by the inherent variability of animal tissues. Any modification of protocol or conditions that increase the size of responses, without undesirable effects on the preparation, decrease measurement errors thereby improving fidelity. Where relatively small changes in resistance are measured, as with ISO in this preparation, magnitude amplification is particularly beneficial. In preparation of dose response curves attention must be paid to the problem of desensitization. No significant desensitization of alpha receptors was found in this preparation. Significant reduction in beta receptor activity with time of exposure to beta agonists has been widely reported (see Lefkowitz, 1978). With this in mind fewer concentrations of ISO were given to each preparation in the construction of dose response curves for this drug. The accuracy of ED50 estimates depends largely upon the slope of the dose response curve. In the case of ISO, addition of 5% human serum to the perfusate had little or no effect upon the slope. The slope of the AD dose response curves was depressed. The reason for this depression is most likely to be the greater enhancement of beta activity resulting in AD dose response curves which have a larger beta component.
Addition of serum to the perfusate caused insignificant changes in ED50 of AD and ISO but gave wider confidence intervals for the estimates. Benefits gained by the addition of serum to the perfusate such as larger absolute response magnitude and more qualitative features such as improved vascular tone, 'autoregulation' (Stainsby, 1964) and preparation viability (Bullivant, 1978a) have to be weighed against the possible loss of accuracy of pharmacological parameter estimates, such as ED50. In this preparation serum addition did not improve the fidelity of pharmacological results, however neither did it alter the conclusions drawn. In vascular preparations where low resting tone is a problem, addition of serum to the perfusate may permit expression of responses such as dilation where otherwise no response could be evoked.

Analysis of the results as either ΔK or $\Delta K\%$ should result in little change in estimates of parameters such as ED50 (Waud, 1975).

In fact the ΔK% ED50 estimates for AD are significantly smaller than the estimates of ED50 from ΔK curves. Similarly for ISO the ΔK% ED50 estimates are also significantly smaller than ED50 estimates for ΔK curves. On the other hand ΔK% ED50 for NAD is larger than the ED50 from the ΔK curve, but not by a significant amount. Why this should be so is unclear since the same data were used in both cases. In a situation such as this the use of transformed data for analysis demands careful consideration (see Latham, 1978).

The baseline pressure differential across the perfused trout trunk is much lower than that measured *in vivo*, (see Wood and Shelton, 1975). The mean pressure differentials across the perfused eel tail were between 1.75 kPa and 2.17 kPa. From section III.3.1 tail pressure differentials in the short-finned eels are between 1.97 and 2.29 kPa in unrestrained unanaesthetised eels of slightly larger size. Martel and Cech (1978) reported pressure differentials across the tail (caudal artery to caudal vein) of anaesthetised and unrestrained conscious winter flounders (*Pseudopleuronectes americanus*), of 2.0 kPa and 2.8 kPa respectively. These values are in excellent agreement, in contrast to dorsal aortic, postcardinal venous pressure differentials of around 3.3 kPa in asiatic eels (Chan and Chow, 1976). The size of the eels used by Chan and Chow was similar to those used for the perfused eel tail. The values given for dorsal and ventral aortic pressures in section III are similar to dorsal and ventral aortic pressures of the asiatic eel (Chan and Chow, 1976). However venous pressures were recorded from different sites in the two reports. Chan and Chow recorded very low venous pressures from the posterior cardinal vein whereas in section III higher venous pressures were recorded from the caudal vein, the point of cannulation for the perfused eel tail preparation. Between the two venous recording sites are the renal and hepatic portal vascular beds which would necessitate a positive pressure at the caudal vein to maintain blood flow through these vital organs. Caudal venous pressures recorded from live fish provide an *a posteriori* rationale for arbitrarily setting the caudal venous pressure of the perfused eel tail to 1.33 kPa for the pharmacological studies. Thus there appears to be no inconsistency between data from these two reports after careful examination of the methods. Likewise it is clear that baseline pressure differentials across the eel tail in intact unanaesthetised eels are in agreement with estimates of the pressure differentials across the same vascular bed in the isolated saline perfused eel tail. This is in distinct contrast with the results from the trout trunk perfusion experiments.

The alpha adrenergic receptors of the eel tail show classical potencies to sympathetomimetic drugs of AD > NAD >> ISO (Ahlquist, 1948; Furchgott, 1967). The mean potency ratio of AD to NAD of ~ 2.78 : 1.00 is very close to that reported for mammalian alpha receptors of 3.2 : 1.0 (Furchgott, 1967), and to those reported in rainbow trout (see Wood and Shelton, 1975; and Wood, 1976).

On examination of the ED50 concentrations for AD and NAD from Wood and Shelton (1975) it is apparent that around ten times the concentration of agonist used for the eel tail is required to elicit a half maximal response from the perfused rainbow trout trunk. The fact that the eel tail is an order of magnitude more sensitive to both AD and NAD is rather surprising. It is also surprising that helical strips of coeliac artery from rainbow trout gave ED50s of 1.1×10^{-8}M (AD) and 2.2×10^{-8}M (NAD), (Holmgren and Nilsson, 1974), which are about one fifteenth of the ED50 concentrations of the perfused trout trunk. Holmgren and Nilsson also gave ED50 concentrations for cod coeliac artery strips of 3.7×10^{-7}M (AD) and 8.7×10^{-7}M (NAD), which are close to those from the perfused eel tail. The poor agreement of data for rainbow trout could simply be the result of the different preparations. Cumulative addition procedures were used in both studies. Restraint should be exercised when comparing results from different preparations from the same species, let alone different species.

Threshold doses of AD and NAD in the eel tail were 10-20 pmoles and 20-50 pmoles respectively. These are similar to threshold doses required to elicit pressor responses from perfused european eel systemic vasculature (Forster, 1976a), but are an order of magnitude higher than thresholds for AD and NAD in the perfused trout trunk (Wood and Shelton, 1975).

Eel tail alpha receptors exhibit significant activity in response to ISO at high concentrations. Systemic pressor responses to ISO in the cod and asiatic eel during beta blockade by propranolol were reported by Helgason and Nilson (1973) and Chan and Chow, (1976). Mammalian alpha receptors exhibit similar behaviour (Jenkinson, 1973). No alpha activity in response to ISO was observed in the trout trunk by Wood and Shelton, (1975) or Wood (1976), despite very high concentrations of ISO (up to 1×10^{-1}M). It is clear that the trout systemic alpha type receptors described by Wood and Shelton (1975) and Wood (1976) are sufficiently different from other fish and mammalian alpha receptors that only limited comparisons should be made.

The beta adrenergic receptors of the eel tail appear to be of the

beta two variety since the potency of ISO >> AD > NAD (Furchgott, 1967). This finding supports the theory that mammalian peripheral beta receptors are homologous to, and phylogenetically derived from the systemic receptors of lower vertebrates (see Wood, 1976). Similarly the beta one type of receptors in the coronary circulation of mammals are homologous to, and derived from the beta one type receptors in fish gills (Keys and Bateman, 1932; Rankin and Maetz, 1971; Wood, 1974a).

The specific alpha antagonist, phentolamine elicited small insignificant decreases in resistance when administered alone. This could be due to blockade of the small component of baseline resistance caused by incomplete washout of alpha agonists.

Phentolamine is a highly specific alpha antagonist in the eel tail preparation. The beta antagonist propranolol is a potent beta antagonist in the eel tail. Reports of significant alpha blockade by propranolol are provided by Kirby and Burnstock (1968) and Holmgren and Nilsson (1974). In the eel tail propranolol controls elicited a small fall in resistance. The qualitative features (time course and shape of trace) suggests alpha blockade rather than partial beta agonistic activity. Partial beta stimulation is common in beta antagonists (see McDevitt, 1977) and is a particular feature of dichloroisoproterenol.

Effective blockade of alpha responses to AD was achieved at agonist to antagonist concentration ratios of around 1 : 40, which is similar to effective ratios in other teleost systemic preparations (see Forster, 1976b). Propranolol produced effective beta blockade at ratios of ISO to Propranolol of around 1 : 20, which is similar to the effective ratio quoted for mammalian beta two receptors (Osnes, 1976). Effective blockade of the beta component of the response to AD was achieved at concentration ratios of AD to propranolol of 1 : 5 to 1 : 10. The response to AD in the eel tail is the sum of opposed alpha constriction and beta dilation. Alpha stimulation by AD near the ED50 during effective beta blockade caused an increase in resistance of 38% when compared to the response to AD without beta blockade. Therefore if the observed responses to AD, (no blockade) are let equal 100% then the response is the result of + 138% (alpha constriction) - 38% (beta dilation).

The eel tail vascular bed has the potential to manipulate vascular resistance by differential stimulation of alpha and beta adrenergic receptors. The next two sections are discussions of how this could be accomplished.

II.6.6.2 Why is the beta dilatory response slower than the alpha constrictory response?

The time course of alpha and beta responses are distinctly different. Perfusion of low concentrations of AD, ($< 1 \times 10^{-7}$M), into the eel tail results in a gradual rise in resistance over the first 180 s. As higher concentrations are infused the time to peak response becomes shorter (see appendix A.21), and the biphasic response becomes more pronounced (see figure II.14a). During infusion, particularly of low concentrations, the development of the alpha response is perfusion limited, that is limited by the rate of delivery to the tissues. Development of the response to NAD is slightly slower and exhibits no small initial peak (see figure II.14c). The decrease to a stable elevated resistance level after the initial peak is attributed to the slower development of the beta response, rather than to overshoot phenomena. It is noted that the records of responses to infusion of AD into the perfused trout trunk (Wood, 1976) do not show this feature. Infusion of ISO produced a decrease in resistance at around 220 s after the start of infusion, and the development of the response was not limited by the rate of delivery of the drug to the tissue. In fact the time taken to reach maximum change in resistance increased slightly as the concentration of ISO is increased. There was no initial peak resistance fall which if present would indicate partial alpha stimulation at concentrations below 1×10^{-5}M. Time zero for infusion experiments was taken as the time when the test solution passed through the bubble trap. The dead space between the bubble trap and the fish vessels was ~ 0.43 mls which would provide a delay of around 27 s, depending on the perfusion rate. Thus the recorded response time in these experiments is in fact longer by some 27 s because of this dead space. Since development of constrictory responses at low concentrations is perfusion limited, and at high concentrations the dilatory response to ISO is masked by constriction, there is little to be gained from a comparison of the response times for infusion of drugs.

Administration of a bolus of 1 nmole is equivalent to perfusion of the preparation with 0.1 mls of 1×10^{-5}M solution. Bolus administration effectively overcomes the problem of perfusion limitation and since by the end of injection all the drug is in the arteries, there is effectively no dead space. Thus it is preferable to compare response time of boli of active agents rather than infused drug concentrations. The differences between the mean response times for all doses of AD and ISO up to 1×10^{-5}M are highly significant ($P < 0.001$, Student's t-test),

the beta dilatory response being slower to develop. Several reports of responses to AD in fish vasculature have shown a rapid alpha constriction (~ 60 s), followed by a slow beta dilatory response (~ 500 s), (see Girard and Payan, 1976; Capra and Satchell, 1974; 1977a).

The reasons for the slower expression of the beta dilation in comparison to alpha constriction may lie either inside or outside the smooth muscle cells. The rate limiting step for the beta dilation could be any one of a number of steps, three of which will be considered here.

The intermediary steps between beta receptor stimulation and muscle relaxation involve cyclic-AMP. Stimulation of beta adrenergic receptors causes elevated c-AMP levels which subsequently act on free intracellular Ca^{++} concentrations thereby inducing tone changes in vascular smooth muscle (Osnes, 1976). Alpha stimulation however apppears to increase membrane permeability to either K^+ or K^+, Na^+ and Cl^- (see Jenkinson, 1973) which directly initiates muscle depolarization (Osnes, 1976; Lefkowitz, 1978). That an additional step involving c-AMP slows the beta response is unlikely since beta mediated ionotropic effects on the rat heart are rapidly developed, yet proceed via the same intermediate steps (Osnes, Refsum, Skomedal and Oye, 1978).

Alternatively within the cells, smooth muscle contractile elements may simply take longer to relax than contract. Restoration of resting (relaxed) intracellular concentrations of Ca^{++} and trans-membrane K^+ and Na^+ gradients could take considerably longer to establish than the sudden influx of ions observed on contraction (Luttgau, 1963).

Extracellularly, the delay in expression of the beta response may be due to the physical separation of the two populations of receptors. For example if alpha receptors were located on the intimal surface of smooth muscle layers and beta receptors were on the adventitial surface, then the alpha and beta responses to circulating catecholamines could be temporarily separated. Holmgren (1978) reported that cod coeliac arteries have adrenergic innervation extending only to the border between the media and adventitia. Stimulation of these nerves elicited both contraction and dilation in the presence of suitable antagonists. Calculations of diffusion times of AD from vessel lumen to medial and adventitial smooth muscle reveals too small a difference to account for the different response times (Wayland and Silberberg, 1978). Thus should an extracellular cause be sought for the response time difference, then an additional barrier to the access of AD to beta receptors must be postulated. If beta receptors were to be found within sympathetic nerve synaptic clefts then a slower beta response would result. Indeed

a slower return to baseline resistance levels after beta dilation would also be explicable. Reports show however that beta receptors form high affinity slowly dissociating complexes with antagonists (Lefkowitz, 1978). This last alternative is supported by the work of Holmgren (1978) on the cod. Holmgren in this paper suggested that beta dilation of cod coeliac arteries may be nerve-mediated.

II.6.6.3 Is teleost systemic vascular tone controlled by sympathetic nerves or circulating catecholamines?

Recent studies reported by Capra and Satchell (1977a,b,c) have shown that elasmobranch fishes possess the ability to manipulate their cardiovascular system by differential release of AD and NAD. When combined with evidence of differential distribution of alpha and beta receptors, this system has sufficient flexibility to regulate cardiovascular parameters within physiological limits. The teleost cardiovascular system was thought to be controlled in a similar manner (see Randall and Stevens, 1967; Randall, 1970; Wood, 1974a), however a number of studies have shown that sympathetic nerves play an important part in regulating blood flow and pressure, especially at the heart (Gannon, 1971; Holmgren, 1977). More recently several reports indicate that normal resting systemic vascular tone may be due to sympathetic nerve activity (see Smith, 1978).

The teleost heart appears to be largely controlled by vagal cholinergic inhibitory and adrenergic excitatory sympathetic nerves. Circulating catecholamines are also believed to play an important part in the regulation of the cod heart (Holmgren, 1977). Teleost gill vascular resistance changes *in vivo* agree well with changes that could be expected from elevated circulating catecholamine levels during say exercise (Wood, 1974a, 1975; Payan and Girard, 1977). The systemic vascular beds of the trout however seem to be under the control of the sympathetic nervous system. On the basis of insensitivity to measured levels of circulating catecholamines in blood, and the absence of significant beta dilatory responses, Wood and Shelton (1975) and Wood (1976) asserted that blood borne catecholamines are likely to play an insignificant part in trout systemic resistance regulation. The discrepancies between ED50s for AD between Wood and Shelton (1975) and Holmgren and Nilsson, (1976) have already been noted. Additional evidence in support of sympathetic control of systemic resistance in trout is provided by the presence of Mayer waves in the rainbow trout (Wood, 1974b). Mayer waves indicate phasic sympathetic activity. Smith (1978) described

experiments on rainbow trout using antihypertensive agents, phentolamine (alpha blocker) and bretilium (sympathetic release blocker). Pretreatment of fish with either the specific alpha blocker, phentolamine, or the adrenergic release blocker, bretilium caused a drop in blood pressure when the fish were subjected to five minutes of strenuous exercise. Normally trout show a marked hypertension in response to such a stimulus (Stevens and Randall, 1967a). The conclusions drawn from these experiments were that the pressor response to swimming for a short period was mediated by sympathetic nerves as both the alpha blocker and the adrenergic release blocker reversed the response. Quite the opposite conclusions were drawn by Wahlqvist and Nilsson, (1977) who subjected cod to alpha (yohimbine), and beta (propranolol), blocking agents and to 6-hydroxydopamine (chemical sympathectomy), and reserpine (adrenergic store depletion). From the results of resting blood pressures after the various drug treatments these authors concluded that the 'adrenergic tonus' that controlled the heart rate and blood pressure in cod was caused mainly by circulating catecholamines rather than sympathetic nerve activity. Smith (1978) has criticised these experiments on the basis of the inappropriate choice of pharmacologically active agents. However in so far as evidence from heavily 'drugged' fish can be accepted, the results from both of these sets of experiments are not mutually exclusive. Since the trout systemic vasculature as described by Wood (1976) exhibits very weak beta dilatory responses to exogenous catecholamines, it is hardly surprising that resistance decreases are accomplished by a reduction in neural tone rather than active beta dilation.

In mammals peripheral resistance is decreased principally by reduction of sympathetic vascular tone. This is accomplished largely by the baroreflex whereby an increase in systemic blood pressure inhibits sympathetic outflow (see Willems and Bogaert, 1978). However sympathetic stimulation of peripheral beta two receptors causes significant dilation with consequent reduction in vascular resistance, especially in muscles (Viveros et al., 1968; Rengo et al., 1976). This response may however be aimed at transcapillary exchange processes rather than resistance (Lundvall and Jarhult, 1976). In addition to reduction in sympathetic tone, sympathetic and parasympathetic cholinergic nerves also cause vasodilation (Mellander and Johannson, 1968). Several other mechanisms for peripheral vasodilation in mammals have been reported such as histamine (Owen, 1977), dopamine (Lang, Bell, Conway and Pudanyl, 1976) and purinergic mechanisms (Su, 1975).

Within teleosts, baroreceptors in the pseudobranchs may reflexly

decrease sympathetic outflow (Laurent, 1967; Laurent and Rousseau, 1972). In trout this is probably a most important cardiovascular homeostatic reflex since resting tone seems to be largely the result of sympathetic nerve activity (Smith, 1978). In fish such as cod and eels there appears to be little resting sympathetic tone and peripheral resistance seems to be maintained by circulating catecholamines. Under these conditions the baroreflex could only be effective through increased activity of sympathetic dilatory nerves. Fish vascular cholinergic sympathetic and parasympathetic nerves elicit vasoconstriction (Burnstock, 1969; Wood, 1977), consequently vasodilation by this route is not possible. Significant purigenic dilation remains a possibility although in the trout ATP caused vasoconstriction (Wood, 1977).

The results of this study of the eel tail vascular bed cannot show whether the beta response is the result of sympathetically released neurohumours or circulating catecholamines, the preceeding discussion however suggests the former. A consideration of how systemic vasodilation might be accomplished in the eel tail by circulating catecholamines is informative.

Increased plasma AD would cause vasoconstriction. Only at very low concentrations ($< 1 \times 10^{-9}$M) could there be dilation in response to AD, and even then it would be very small. After the constriction, partial dilation by direct beta stimulation could represent autoregulatory escape from vasoconstriction as observed in mammals (Lundvall and Jarhult, 1978). Within the eel tail there could be differential distributions of alpha and beta receptors as seen in dogfish (Capra, 1975). If alpha receptors were primarily located in white muscle vasculature and beta receptors in the red muscle vasculature then increases in plasma AD levels would redirect blood from the white muscles to the red muscles. Given that red muscles are more active during sustained swimming this hypothesis is not unreasonable. This might also explain the slow release of lactate into blood observed in exercising fish (Driedzic and Kiceniuk, 1976). Stevens however reported no significant redistribution of blood in the musculature of the swimming trout. If beta receptors were present in low concentration in the white muscle vasculature, then the dilatory responses of the eel tail would be largely the result of red muscle vessel dilation only. Despite the fact that red muscle contains about three times the blood volume per gram than white muscle, the sheer bulk of the white muscles means that over 96% of the vascular volume of the tail resides in the white muscle. The magnitude of the dilatory responses of the eel tail

to ISO and AD in the presence of an alpha blocker make this extremely unlikely. Clearly there is no significant differential alpha and beta receptor distribution between the red and white muscles of the eel tail. From this brief consideration of beta stimulation by circulating catecholamines it seems unlikely that dilation of the tail vessels of the eel could be efficiently accomplished by circulating catecholamines.

To summarise, it is apparent that in trout there is significant positive sympathetic vasomotor tone in the systemic vasculature and that alteration of the level of sympathetic tone is the principal mechanism whereby systemic resistance adjustments are made. In eels and perhaps cod, circulating catecholamines play an important role in the maintenance and regulation of systemic vascular tone. Increases in resistance are probably the result of alpha stimulation by blood borne catecholamines and decreases in resistance may be the result of sympathetic nerve activity.

II.7 Blood Volume and Capacitance Responses in Vascular Beds

II.7.1 Introduction

The vascular volume of fish has been estimated by many authors. Holmes and Donaldson (1970) provide an extensive review of the early literature. More recently refer to Avtalion, Gorlin, Gutwirth and Wojdani (1974). Two aproaches are regularly employed to determine vascular volume. The first involves dilution of the formed elements or cells in the blood by a known amount of isotonic saline (cell dilution). The second utilizes the distribution of a marker in the plasma. Since blood consists of cells and plasma each method corrects for the volume of cells or plasma not measured using the haematocrit. Cell dilution methods give lower values for total blood volume than plasma marker methods. For a discussion of the problems involved in the two methods see Gregerson and Rawson (1959). Levick and Michel (1973) have shown that in the presence of albumin, the common plasma marker Evans Blue move through frog capillary walls at approximately twice the rate of the dye alone. Binding of the dye to the vessel walls will also lead to over-estimates of blood volume by this method. Significant quantities of Evans blue do bind to vessel walls according to Fry et al., (1977). Dye is found in the lymphatic fluid in concentrations near that found in the blood and will have similar effects to the measurements of blood volume as extravasation of Evans blue.

Fish blood volumes are reported to be larger in cyclostomes than in elasmobranchs. Within the bony fishes, Chondrostei and Holostei appear to have larger blood volumes than Teleostei (Thorson, 1961; Holmes and Donaldson, 1970). Randall (1970) gave a mean blood volume for all teleost species measured of around 3 mls per 100 g body wt (3%). Estimates of blood volume should use both methods to arrive at a volume near the true volume, and an accuracy of ±5% is the best that can be expected.

The distribution of blood throughout the body has been estimated before and after exercise in the Rainbow trout by Stevens (1968). Stevens reported minimal changes in distribution between the two conditions. In agreement with Gorkiewicz (1948), Stevens found that lateral red muscle contained two to three times more blood per gram than white muscle and that about 60% of the total blood volume was contained within the large vessels.

In mammals veins are largely responsible for vascular volume changes (see Oberg, 1967; Mellander and Johansson, 1968). Less than 10% of the blood volume in a vascular bed is contained in the resistance vessels (arterioles). However these small vessels produce about 80% of the resistance adjustments. The maximum volume change that could be accomplished by precapillary vessel diameter changes, assuming a four-fold increase in resistance, is less than 5% of the blood volume of the vascular bed (Mellander, 1960; Oberg, 1967). Measurements of volume changes (Mellander, 1960; Rose, Kot, Cohn, Freis, and Eckert, 1962; Oberg, 1967) revealed that this represents only 10% of the recorded changes. These results indicate that up to 90% of the capacitance changes originate from alterations in venous tone. Fish veins are reported to be aneural and lack smooth muscle (Burnstock, 1969). Consequently capacitance changes in a fish vascular bed should be the result solely of precapillary vessel diameter changes. Such changes should also be small, less than 5% of the vascular volume of the vascular bed. Capacitance effects as discussed here are those resulting solely from changes in vessel radius. Alterations in tissue fluid volumes and pressures which could have appreciable effects upon vascular volume are considered in section II.7.5.

II.7.2 Vascular volume of the perfused eel tail

Vascular volume of the tail was measured during perfusion by two separate methods; dilution of red blood cells and by inulin dilution.

II.7.2.1 Red blood cell vascular volume of the perfused eel tail

Three perfused eel tails were prepared in the normal manner. The blood that was collected after heparinisation was stored at $4^{\circ}C$ and allowed to settle. During the first hour of perfusion with basic saline at a venous pressure of 1.33 kPa the efficiency of perfusion was measured several times. The preparation was then perfused with the blood cell suspension in ringer and the effluent from the caudal vein replaced into the reservoir. The reservoir was continuously and vigorously bubbled with 95% O_2/5% CO_2 which stopped cell settling. This type of system is a simple closed two compartment model with exchange in both directions (see Shippley and Clark, 1972). The two compartments will eventually reach equilibrium and from the change in concentration in the reservoir and its initial volume, the volume of the other compartment is calculable. Haematocrit (concentration), was measured at time zero,

1 min, 2, 5, 10, 15, 20 30 minutes in duplicate 10 µl microhaematocrit tubes and centrifuged at 2600 rpm for 5 minutes. In the analysis of the results the loss of perfusate from the preparation, or leakage (l) must be taken into account. The concentration of cells in the fluid leaked from the preparation is assumed to be the same as the concentration in the vasculature. Figure II.24a illustrates the model that applies to this system.

Total cells initially = total cells finally

$$H_i.Y_i \qquad H_i.Y_i = H_f(X + Y_f) + \text{cells lost}$$

$$\text{Cells lost} = \int_0^t l.\ H'_t.dt$$

Where H_i = initial haematocrit

Y_i = initial reservoir volume

H_f = final haematocrit

X = vascular volume

Y_f = final reservoir volume

l = leakage rate

H'_t = haematocrit in the vessels

To estimate the cells lost, H'_t is assumed to be the reflection of H about the equilibrium concentration. Mean H'_t is calculated from this curve and when multiplied by the leakage rate, l, gives the cells lost and allows the computation of the vascular volume, X.

A graph of the reservoir haematocrit against time is presented in figure II.25a, with the corresponding assumed H'_t, haematocrit of the vascular cell suspension. From these graphs it is clear that $H = H'_t$ at or before 20 minutes. Thus 20 minutes was chosen as the final or equilibration time. Calculations of tail vascular volume by this method are presented in table II.8.

II.7.2.2 Inulin vascular volume of the perfused eel tail

After the determination of the red blood cell volume in each of the tails, at least two hours was allowed to remove any of the cells still remaining in the vessels. A solution of ^{3}H-radiolabelled inulin (Radiochemical Centre, Amersham, England), was then perfused through the tail in the same manner. ^{3}H-inulin concentration in the reservoir was measured at 0, 1, 2, 5, 10, 20, 30, 40, 50 and 60 minutes by removing 50 µl and counting the disintegrations per minute in a Unilux liquid

FIGURE II.24a Diagram of simple two compartment model for estimating vascular volume of the perfused tail. From the initial and final volumes and red blood cell concentrations the vascular volume can be calculated. Leakage was estimated from the change in reservoir volume with time.

Y reservoir volume

X vascular volume

l leakage rate from the preparation

FIGURE II.24b Multi-compartment model for vascular and extravascular spaces in the eel tail. From the information available it is not possible to estimate the sizes of all the compartments, however movement of ^{3}H-inulin into the interstitial, lymphatic and cellular spaces was slow relative to the vascular compartment enabling estimates of vascular volume to be made in a similar manner to the red blood cell method.

Y volume of the open external reservoir

X vascular volume

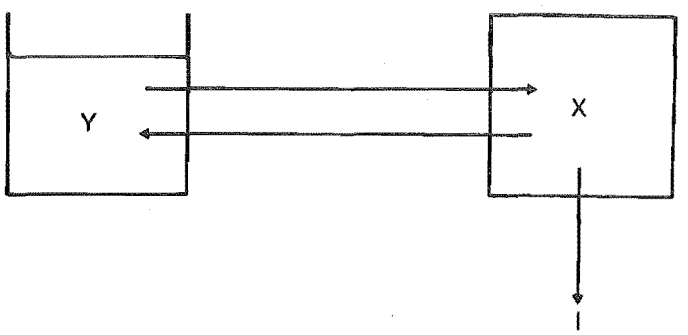
I interstitial volume

L lymphatic volume

C cellular volume

l leakage rate from the preparation

a



b

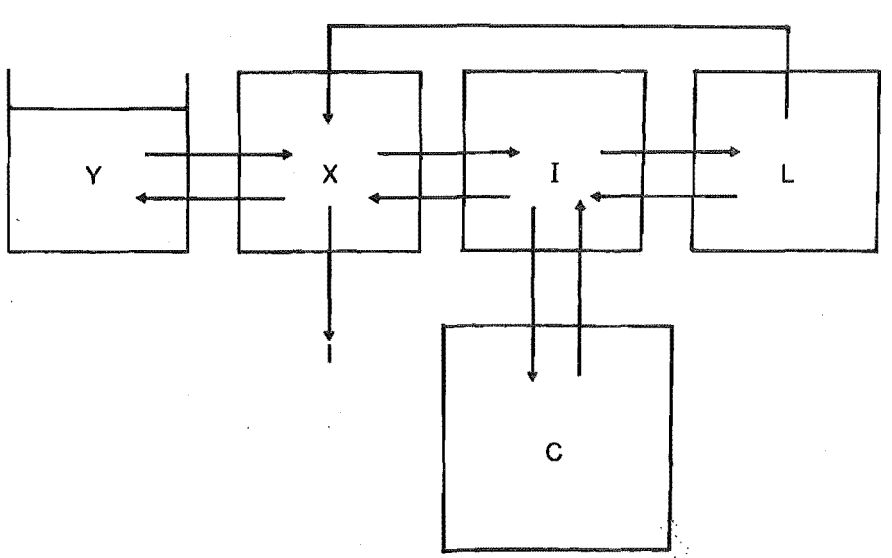


FIGURE II.25a Graph of external reservoir red blood cell concentration (haematocrit) versus time during short circuit perfusion. Little change in haematocrit was observed after 10 minutes of perfusion indicating equilibration of red blood cells between the vascular space and the external reservoir. Amount of cells lost due to leakage was estimated from the leakage flow rate and the haematocrit of the vascular fluid. Haematocrit of the vascular fluid was assumed to be the reflection of the reservoir haematocrit about the equilibration haematocrit.

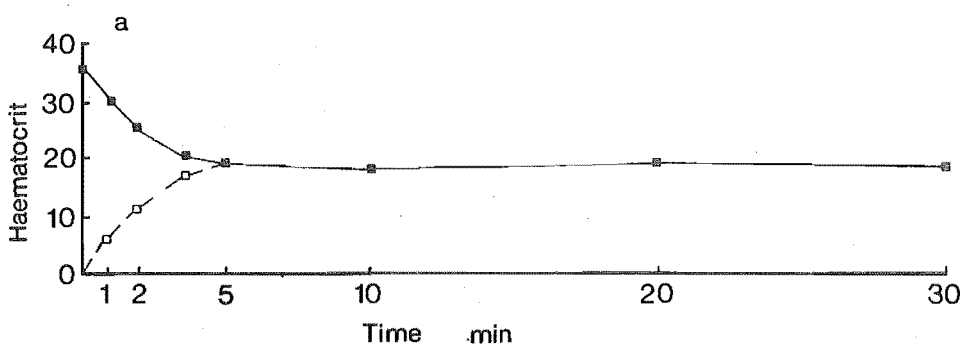
-■- reservoir haematocrit

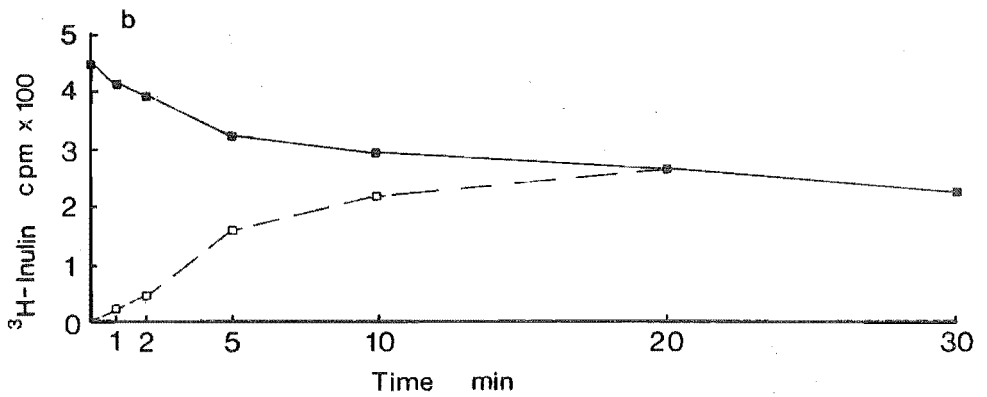
-□- assumed vascular haematocrit

FIGURE II.25b Graph of ^{3}H-inulin concentration versus time during short circuit perfusion. Equilibration of ^{3}H-inulin between the tail and external reservoir was slower than red blood cells, however after 20 minutes vascular equilibration was assumed to be complete and the fall in reservoir ^{3}H-inulin concentration thereafter probably reflects movement of ^{3}H-inulin into compartments other than the vascular space (see figure II.24b). Vascular ^{3}H-inulin concentration and H-inulin vascular volume of the tail were estimated as in the red blood cell method. Mean vascular volume of three perfused eel tails estimated by these two methods was 3.1 ml per 100 g tail tissue weight (see table II.8).

-■- reservoir ^{3}H-inulin concentration

-□- vascular ^{3}H-inulin concentration


a
Haematocrit
40
30
20
10
0
1 2 5 10 20 30
Time min


b
^{3}H-Inulin cpm x 100
5
4
3
2
1
0
1 2 5 10 20 30
Time min

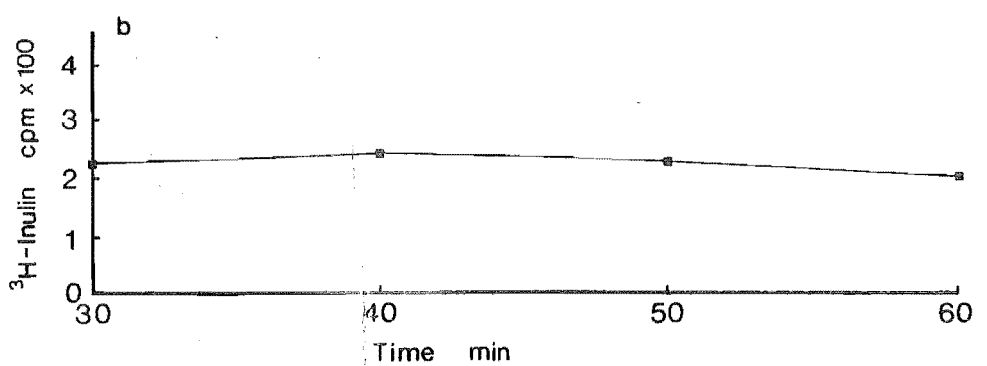

b
^{3}H-Inulin cpm x 100
4
3
2
1
0
30 40 50 60
Time min

Table II.8 Vascular volume of the isolated saline perfused eel tail as measured by red blood cell dilution and ^{3}H-inulin dilution (for details see text).

Fish number	Red blood cell dilution vascular volume ml/100 g tail wt	^{3}H-inulin dilution vascular volume ml/100 g tail wt
1	3.13	4.48
2	2.61	2.95
3	2.75	2.47
Mean vascular volumes	2.83 ± 0.15	3.30 ± 0.60

Overall vascular volume = 3.065 ± 0.298

scintillation counter for 60 seconds. The size of the reservoir was minimized to ensure large changes in concentrations yet had to be large enough to last for the required time. The volume needed was between 5 and 15 ml; the greater the leakage rate and the larger the tail, the larger the reservoir volume required.

The raw counts were used to construct the graphs of ^{3}H-inulin concentration against time (see figure II.25b), and an approximation of the ^{3}H-inulin concentration in the vascular compartment was drawn for these graphs as in the red blood cell dilution analysis. The graph of ^{3}H-inulin concentration versus time showed, not surprisingly, a slow uptake of inulin into a non-vascular compartment or compartments. From the model outlined in figure II.24b, it is clear that there is insufficient information available to completely analyse the inulin spaces. The information from these experiments can however be used as a check on the vascular volume obtained by cell dilution.

Analysis of these data was carried out in a manner somewhat similar to the red blood cell dilution, except that a further correction for the loss of ^{3}H-inulin into the 'slow' compartment, whatever its nature, had also to be made. The loss of ^{3}H-inulin to the slow compartment was estimated from the semilog plot of the ^{3}H-inulin concentration against time which allows extrapolation back to time zero provided that the two compartments have markedly different time courses, (Shippley and Clark, 1972). Thus the equation for the ^{3}H-inulin in the system is

$$[^{3}HI]_{i}.Y_{i} = [^{3}HI]_{f}\ (X + Y_{f}) + \int_{o}^{t} {}^{3}HI_{t}.dt + {}^{3}HI \text{ in slow cpt},$$

where square brackets represent concentrations and all other symbols are the same as for the red blood cell dilution analysis.

The volume of the delivery system (1.287 ml) was subtracted from values of the volume X from the inulin dilution and the red blood cell dilution methods. The results in table II.8 are expressed as mls per 100 g tissue weight and provide a working figure of 3% of tissue weight for the vascular volume. This is very close to most of the previously reported values.

II.7.3 Changes in vascular volume of the isolated perfused eel tail in response to catecholamines

Changes in the vascular volume of isolated perfused tissue preparations have been measured by several means, (marker transit times, plethysmography, changes in volume of extracorporeal reservoir). Perfusion at constant inflow while simultaneously measuring the outflow rates allows calculation of the fraction of perfusate 'lost'. Once the efficiency of a perfused eel tail has stabilised then it is remarkably constant throughout the life of the preparation, (± 2%). Lower outflow rates should indicate an increase in vascular volume (dilation) whereas higher outflow rates should indicate an expulsion of vascular fluid as would occur during vasoconstriction. Such changes should be simultaneous with resistance changes since the fluid is incompressible, although vascular compliance may accommodate some of the volume changes.

Complete outflow records are available for 39 preparations. Outflow represents about 86% of the fluid passing through the vascular bed and as a consequence volume adjustments calculated from them will be conservative. Graphs of mean outflow changes with time during infusion of different concentrations of AD and ISO are presented in figures II.26 and II.27 respectively, (see also appendices A.32 (AD), and A.33 (ISO)).

The vascular volume change is calculated from the sum of the outflow changes that occur during resistance increase or decrease. Percentage vascular volume changes are given on figures II.26-II.29.

Calculated vascular volume changes in the eel tail are small. Constriction is the dominant response to catecholamines in the systemic vasculature. The largest volume decrease during constriction was observed when 1×10^{-7}M AD was administered. The volume change represents a decrease of about 1.4% of the tail vascular volume. This is considerably smaller than the 30% decrease reported by Mellander (1960) and Rose et al. (1962).

Bolus administration of drugs produced a pronounced increase in

FIGURE II.26 Changes in venous outflow with time during infusion of adrenaline at concentrations between 1 x 10^{-8}M and 1 x 10^{-3}M. Estimated % changes in vascular volume (V) were based on a tail vascular volume of 3 ml per 100 g tail weight and are the sum of the outflow changes during resistance changes. Estimates of % changes in interstitial volume (I) were based on a tail interstitial volume of 10 ml per 100 g tail weight and are the sum of changes in outflow after resistance changes. Vasoconstriction, by definition implies a decrease in vascular volume, therefore it is logical to view a decrease in outflow during vasoconstriction as extravasation of fluid, that is an increase in interstitial volume rather than an increase in vascular volume. Consequently changes in vascular and/or interstitial volumes have been calculated only where appropriate. Mean initial outflow and mean changes in outflow during infusion of adrenaline are tabulated in appendix A.32. Arrows indicate times of peak resistance responses.

a. 1 x 10^{-8}M adrenaline. $\Delta V = -0.78\%$

b. 1 x 10^{-7}M adrenaline. $\Delta V = -1.36\%$

c. 1 x 10^{-6}M adrenaline. $\Delta V = -0.314\%$
$\Delta I = +0.123\%$
This concentration was the only one in which the two components of the outflow change were clearly delineated.

d. 1 x 10^{-5}M adrenaline. $\Delta I = +1.57\%$

e. 1 x 10^{-4}M adrenaline. $\Delta I = +2.78\%$
Histogram on the abcissa of the graph indicates the number of preparations 'swimming' (see right hand ordinate, n = 9).

f. 1 x 10^{-3}M adrenaline. $\Delta I = +0.372\%$
Histogram on abcissa of the graph indicates the number of preparations 'swimming' (see right hand ordinate, n = 6).

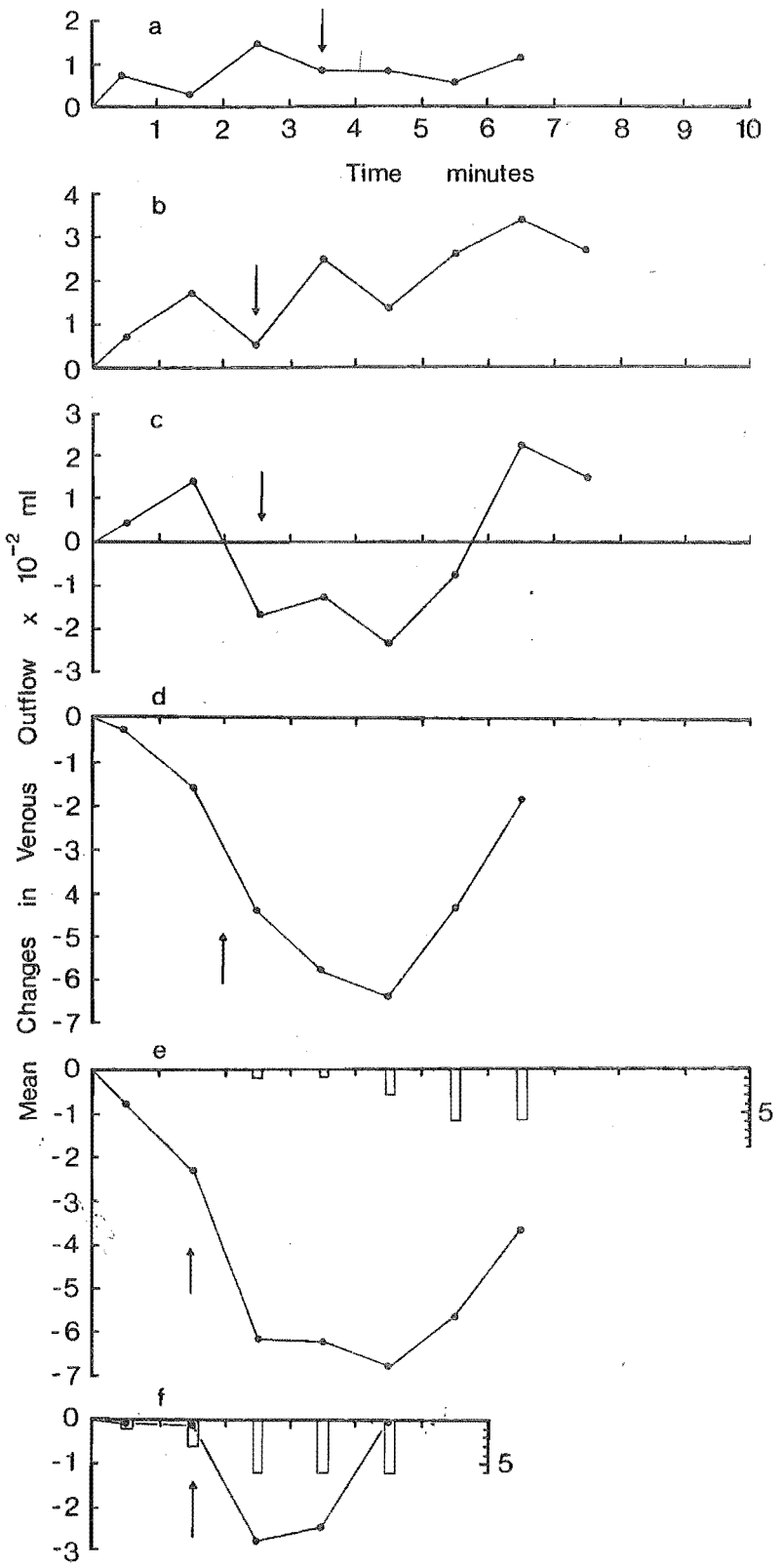

a
b
c
d
e
f
Time minutes
Mean Changes in Venous Outflow x 10^{-2} ml
5
5

FIGURE II.27 Changes in venous outflow versus time during infusion of isoprenaline at concentrations between 1×10^{-9}M and 1×10^{-3}M. Estimated % changes in vascular volume were based on a tail vascular volume of 3 ml per 100 g tail weight and are the sum of outflow changes during resistance adjustments. Increases in vascular volume occurred during vasodilation (1×10^{-9}M to 1×10^{-6}M isoprenaline) and decreases in vascular volume occurred during constriction (1×10^{-4}M and 1×10^{-3}M isoprenaline). Mean initial outflow and changes in outflow are tabulated in appendix A.33. Arrows indicate times of peak resistance responses.

a. 1×10^{-9}M isoprenaline. $\Delta V = +0.651\%$

b. 1×10^{-8}M isoprenaline. $\Delta V = +0.870\%$

c. 1×10^{-7}M isoprenaline. $\Delta V = +0.783\%$

d. 1×10^{-6}M isoprenaline. $\Delta V = +0.634\%$

e. 1×10^{-5}M isoprenaline. $\Delta V = -0.521\%$

f. 1×10^{-4}M isoprenaline. $\Delta V = -0.499\%$

g. 1×10^{-3}M isoprenaline. $\Delta V = -0.596\%$

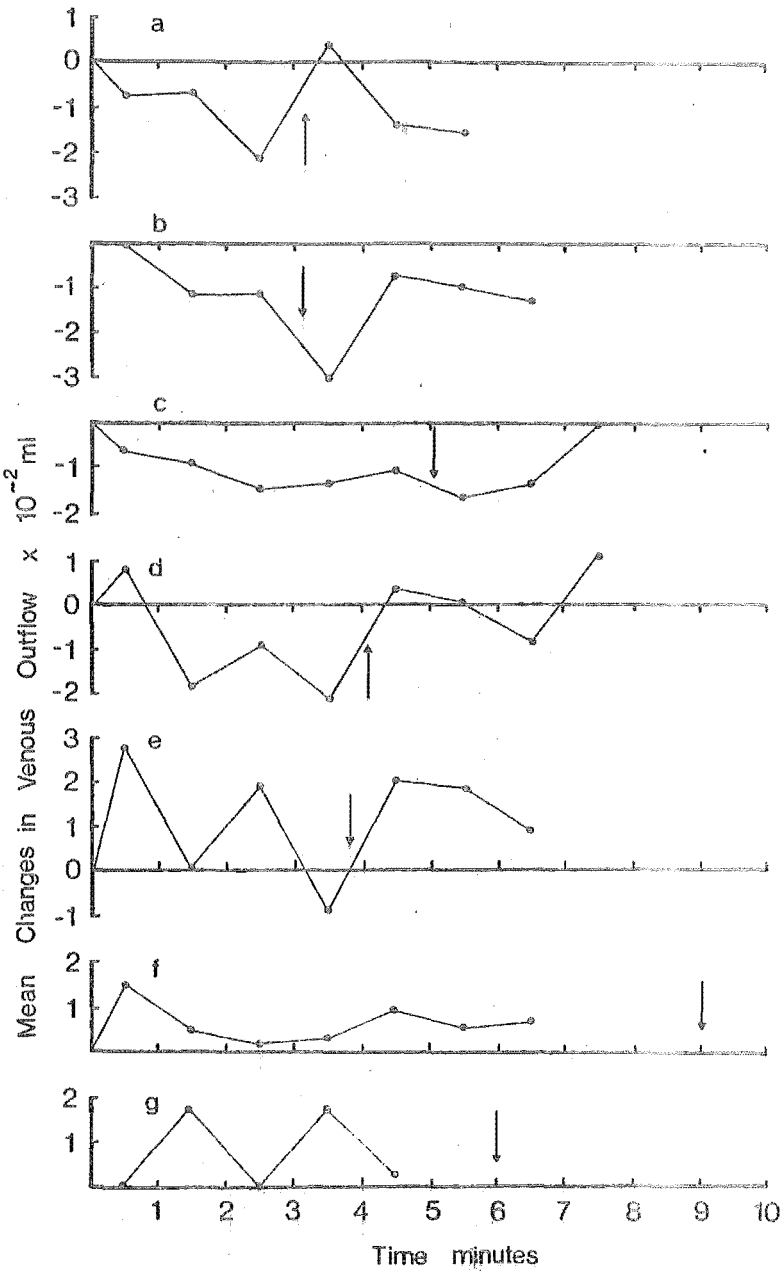

a
b
c
d
e
f
g
1
0
-1
-2
-3
3
2
Mean Changes in Venous Outflow x 10^{-2} ml
1 2 3 4 5 6 7 8 9 10
Time minutes

FIGURE II.28 Changes in venous outflow with time after injection of 0.5 - 100 nmoles of adrenaline. Changes in venous outflow due to the 0.1 ml vehicle have been accounted for in the calculation of changes in vascular volume. Estimates of % changes in vascular and interstitial volumes were calculated as previously explained (see figure II.26 and II.27). Mean initial venous outflow and mean changes in outflow for blanks and all doses of adrenaline are tabulated in appendix A.34. Arrows indicate times of peak resistance responses.

a. 500 pmoles (0.5 nmoles) adrenaline. $\Delta V = -0.575\%$
$\Delta I = +0.0123\%$

b. 1 nmole adrenaline. $\Delta V = -0.293\%$
$\Delta I = +0.278\%$

d. 10 nmoles adrenaline. $\Delta V = -0.351\%$
$\Delta I = +1.34\%$

e. 100 nmoles adrenaline $(\Delta V = +0.0564\%)$
$\Delta I = +5.95\%$

Note that during vasoconstriction in response to 100 nmoles adrenaline extravasation of fluid was greater than the decrease in vascular volume expected with vasoconstriction. This will result in an underestimate of the change in interstitial volume.

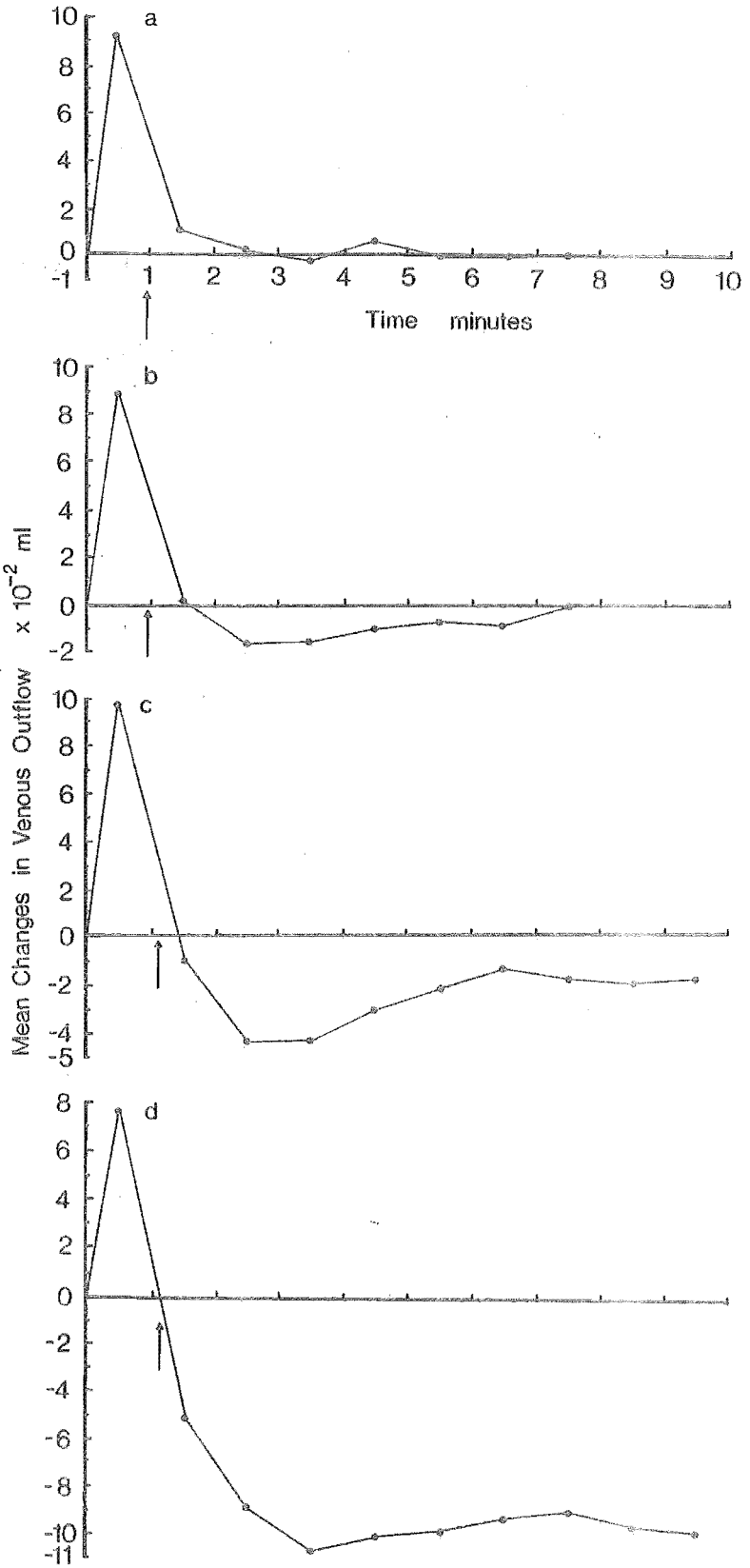

a
b
c
d
Time minutes
Mean Changes in Venous Outflow x 10^{-2} ml

FIGURE II.29 Changes in venous outflow with time after injection of 0.5 - 100 nmoles of noradrenaline. Changes in venous outflow due to the 0.1 ml vehicle have been accounted for in the calculations of changes in vascular volumes. Estimates of changes in vascular and extravascular volumes were calculated as previously explained (see figure II.26 and II.27). Mean initial venous outflow and mean changes in outflow for blanks and these concentrations are tabulated in appendix A.35. Arrows indicate times of peak resistance responses.

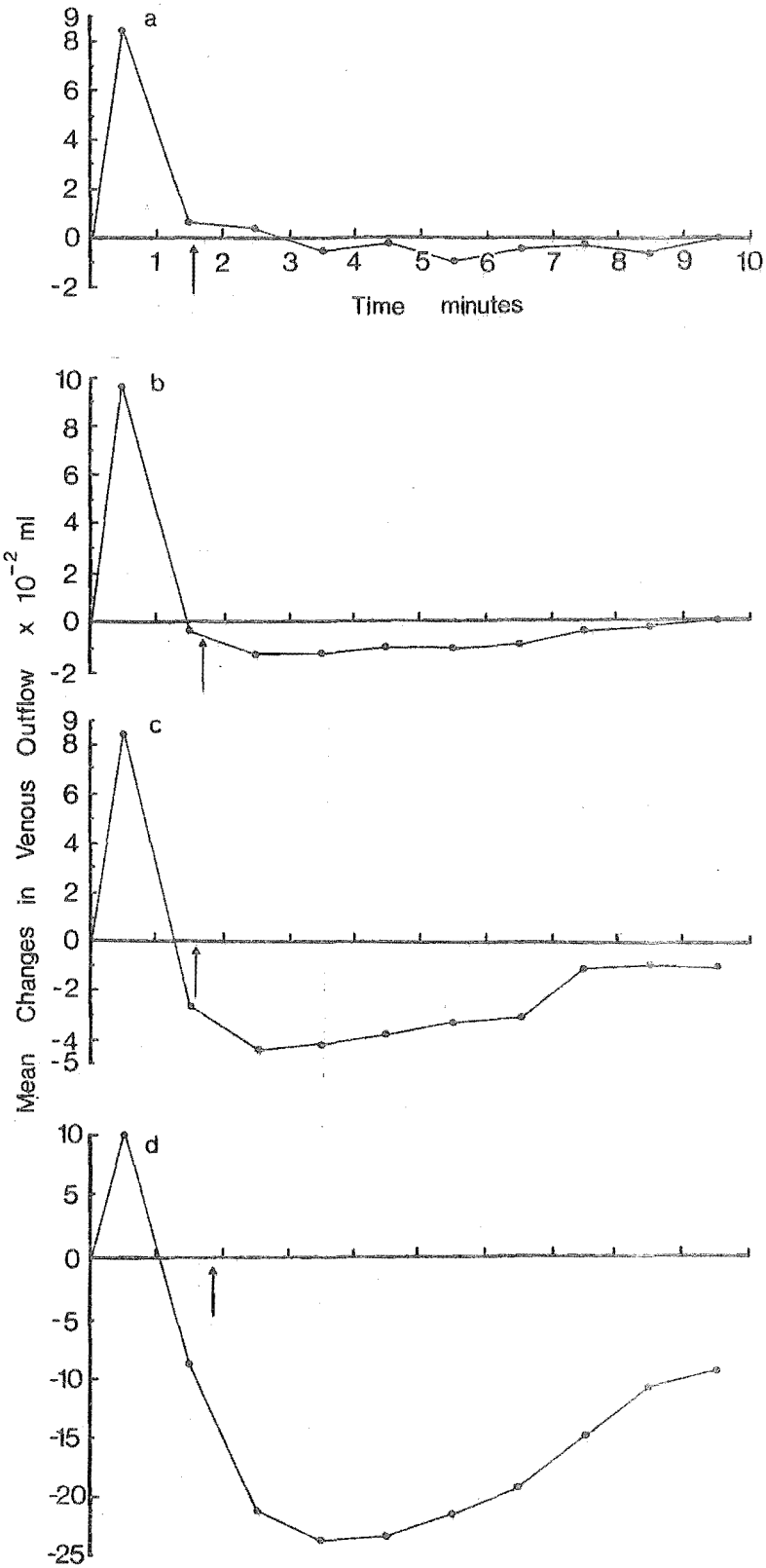
a. 500 pmoles (0.5 nmoles) noradrenaline. $\Delta V = -0.822\%$
$\Delta I = +0.218\%$

b. 1 nmole noradrenaline. $\Delta V = -0.818\%$
$\Delta I = +0.589\%$

c. 10 nmoles noradrenaline. $\Delta V = -0.511\%$
$\Delta I = +1.73\%$

d. 100 nmoles noradrenaline. $\Delta V = -0.932\%$
$\Delta I = +10.57\%$

Note that during vasoconstriction in response to 100 nmoles extravasation was not as great as the decrease in vascular volume as was the case for this dose of adrenaline. Note also that the ordinate scale is half that of the above graphs.


a
b
c
d
Time minutes
Mean Changes in Venous Outflow x 10^{-2} ml

outflow when the 0.1 ml bolus was injected into the preparation, (see figure II.18c). Blank injections of 0.1 mls of saline, without any active agents, were delivered throughout all experiments and have been used to correct for the change in outflow due solely to the vehicle for each set of experiments. Graphs of mean outflow changes with time after injection of different doses of AD and NAD are presented in figures II.28 and II.29 respectively, (see also appendixes A.34 (AD), A.35 (NAD), and A.36 (ISO)).

The maximum decrease in vascular volume when a bolus of AD or NAD caused vasoconstriction was 0.8% for a dose of 500 pmoles NAD. Since all bolus injections of AD or NAD resulted in a decrease in outflow after 60-180 seconds, the positive values during this short initial period were summed to estimate decreases in capacity. The peak resistance response times are indicated on the graphs and were around 1 to 1.5 minutes for all bolus doses of AD and NAD analysed.

Isoprenaline caused vasodilation in the eel tail. Thus we could expect a decrease in outflow whilst this dilation occurred. When ISO was infused into the preparations there were consistent, but small increases in vascular volume of around 0.73 ± 0.06% for the four concentrations that produced the most marked dilations (see figure II.27). Administration of ISO as a bolus revealed no clear trend in outflow adjustment. All the volume changes were small (< 1%) there being increases in vascular volume at three concentrations (20 pmoles, 200 pmoles, 100 nmoles) and decreases in volume at the other six intermediate concentrations tested (see appendix A.36).

II.7.4 Discussion of vascular volume changes in the isolated perfused eel tail

These results confirm that an increase in vascular resistance is accompanied by a decrease in vascular volume. Conversely a decrease in resistance is often accompanied by an increase in vascular volume. The magnitude of these changes, as measured here, are easily explained in terms of changes in radius of precapillary resistance vessels and require no capacitance functions of veins for their explanation.

It is clear from these studies that although the white muscles of the tail of an eel contains a large volume of blood (around 70% of total vascular volume) it is not readily mobilised by catecholamines under these conditions. Teleosts have a small blood volume per body weight compared to other vertebrates (Thorson, 1971). Mobilization of this large volume of blood might be expected when increased circulatory

efficiency is required, for example during swimming. These results suggest that this could not be accomplished by elevated blood catecholamine levels in the short-finned eel.

II.7.5 Extravasation of fluid and lymph formation in the perfused eel tail in response to catecholamines

II.7.5.1 Introduction

Capillary filtration and resorbtion processes are significantly affected by resistance adjustments through changes in capillary pressures and capillary filtration coefficient (see Pappenheimer and Soto-Rivera, 1948 and section I.1.1). Increases in arterial pressure during vasoconstriction will increase mean vascular pressure and result in greater ultrafiltration of fluid from the capillaries. Greater vascular pressure will be transmitted through the interstitium to the lymphatics causing higher lymphatic pressures to prevail (Granger and Taylor, 1978). Increased capillary permeability in response to AD and NAD (Payan and Girard, 1977; Reichel, 1977), will also increase ultrafiltration of fluid from within the capillaries. Thus during vasoconstriction in response to AD and NAD it seems likely that the Starling equilibrium at the capillaries is displaced and interstitial fluid formation occurs. Furthermore AD not only raises arterial pressure and increases capillary permeability but also has been reported to increase the measured size of the interstitial space in many animal tissues, including teleosts (Gras, et al., 1971; Isaia, Maetz and Haywood, 1978; see also Vaccari and Maura, 1978).

Analysis of the outflow data revealed that following the initial vascular volume adjustment there was a decrease in outflow when high doses of AD and NAD were given (see figures II.26, II.28 and II.29). This effect was more pronounced when boli of drugs were administered. No consistent trend was observed for ISO. This secondary decrease in outflow could be due either to extravasation of perfusate or redirection of the perfusate through the vessels in the neural arch which provided most of the leakage. The near constant value for efficiency before and after drug administrations, and throughout the life of a preparation argue against the latter. The arguments outlined above strongly suggest that the decrease in outflow from the preparation after the peak or steady elevated resistance has been reached in response to high concentrations or doses of AD or NAD indicates movement of perfusate into the interstitial space.

The clearly different time course and different directions of the fast vascular volume adjustment and slower extravasation of fluid allows separation of the two effects. The facts that outflow reflects both vascular and extravascular volume adjustments, and that the two components of the outflow change act in opposite directions during constriction means that for the time when the two effects overlap, outflow rate will give smaller estimates of volume changes in the two compartments. That the two components are temporarily separated is fortuitous in that it allows calculation of each.

Measurements of interstitial space in fish are sparse. Holmes and Donaldson (1970) provide figures for an 'average' teleost whole body interstitial space of around 12%. This value is calculated from the sucrose (extracellular) space minus the vascular space. Chan, Chester-Jones, Henderson and Rankin (1967) gave values for the sulphate space in fresh water eel tongue and parietal muscle. The sulphate space of tongue muscle was 10.5 ± 0.5%, and for the parietal muscle, 4.8 ± 0.4%. The size of this compartment was not measured in the perfused eel tail but for the purposes of these experiments will be taken as 10 ml per 100 g tissue, (10%).

II.7.5.2 Results

Interstitial volume changes were calculated from the sum of outflow changes that occurred after resistance adjustments had ended. Percentage interstitial volume changes are given with figures II.26 - II.29.

Continuous infusion of 1 x 10^{-4}M AD produced a retention of 0.32 ml of fluid over the first seven minutes. This is equivalent to an increase of ~ 2.7% in the extravascular extracellular space and was the largest change recorded from continuously infused preparations. Although a steady elevated resistance was reached within 60 to 120 s the outflow fell to a minimum at 250 to 300 s. After this minimum there was a steady trend back toward outflow values recorded before introduction of the drug. This return to resting outflow rates appears to be assisted by the gross muscular movements that occurred in response to 1 x 10^{-4}M and 1 x 10^{-3}M AD (see figures II.26e and II.26f). In the case of 1 x 10^{-3}M AD after three minutes all (6) preparations were 'swimming'. At AD concentrations of 1 x 10^{-6}M and 1 x 10^{-5}M the return toward the baseline outflow levels was seen within 5 minutes of the start of infusion in the absence of any swimming movements.

Injections of AD and NAD of 1, 10, and 100 nmoles all resulted in secondary decreases in outflow. The magnitude of the falls in outflow were rather higher in the case of bolus injections than with continuous infusion. Larger estimates of the increase in interstitial volume result. Injection of 100 nmoles of AD caused an estimated 5.9% increase over ten minutes, and 100 nmoles NAD caused a 10.5% increase over the same time. In all but one case of injection of 1, 10, 100 nmoles AD and NAD there was a trend back toward preinjection outflow levels four to five minutes after administration. With bolus injection it must be remembered that the drug is continuously being washed out from the preparation and so the response should be expected to diminish.

II.7.5.3 Discussion

From the literature on transcapillary fluxes and factors that affect these fluxes it is clear that during vasoconstriction, an increase in interstitial fluid volume could be expected. Increased weight of perfused tails after perfusion with vasoconstrictory drugs provides some support for this notion. The time lag between peak arterial pressure and peak outflow change was 60-120 s. A similar time lag of 90 s between arterial pressure peak and interstitial fluid build up was recorded by Poole (1977) in isolated perfused dogfish gills after alloxan administration. Alloxan is believed to increase capillary permeability (Goetzman and Vischer, 1969). Thus it seems reasonable that the observed outflow changes reflect extravasation of perfusate.

About five minutes after AD or NAD infusion or injection there was a trend back toward pre-administration outflows. This probably represents equilibration of the vascular bed to a new set of capillary exchange conditions whereby higher interstitial pressure increases resorption at the venous end of the capillaries (Jacobsson and Kjellmer, 1964). Swimming movements of the tail with high concentrations and doses of AD and NAD probably assist interstitial fluid recovery (Gnepp and Sloop, 1978). Although a trend toward greater fluid retention with increased AD concentration is apparent only a small fall in outflow was noted at 1×10^{-4}M and 1×10^{-3}M AD. These small changes in outflow do not necessarily imply less extravasation of perfusate. Muscular contractions increase venous return in elasmobranch fish (Satchell, 1965; Birch, Garre, and Satchell, 1969). It is clear that body-movements augment interstitial as well as blood circulation in the tails of fishes.

At elevated capillary pressures and transcapillary flows when the

interstitial pressure and space is increased, more lymphatic fluid would be formed (McMaster, 1946). Increased lymph production should be reflected in increased lymph heart activity. Increased frequency and/or strength of contraction could be expected as a response to the greater volume of lymph to be handled. Increased venous capillary resorbtion and the capacity of the interstitium to buffer the effects of increased extravasation of fluid mean that changes in lymphatic flow will be considerably smaller than changes in perfusate efflux from the capillaries. However the effects on the lymph heart should occur soon after the fall in outflow and should occur only when relatively large changes in interstitial fluid volume are recorded.

II.8 The Effects of AD, NAD and ISO upon Lymph Heart Frequency and Amplitude

II.8.1 Introduction

Translocation of body fluids in the tails of fish appears to have presented problems. Pumps other than the true or branchial heart have emerged in all fish classes. The portal venous heart of lampreys (*Myxine* spp.) (see Fange, Bloom and Ostlund, 1963) and the venous tail pump in certain elasmobranchs (Birch, Carre and Satchell, 1969) are examples of accessory pulsatile structures involved in blood circulation (see section I.6.2). In addition to these specific mechanisms, skeletal muscle contraction assists venous return to the heart (see Carlsten and Grimby, 1966).

Caudal lymphatic hearts actively pump lymph from collecting vessels into the blood stream by muscles specifically assigned to this role. Whether the fluid pumped by the caudal heart was blood or lymph was disputed in the early reports, however Hyrtl (1843 cited in Kampmeier, 1969) resolved the question and subsequent reports clearly established that they do pump lymph (Wharton-Jones, 1868; Favaro, 1905, 1906). From these accounts it is clear that one function of the lymph heart is to pump lymph, but the significance of this rather small organ in this role has never been investigated. The close proximity of the urophysis, which secretes hormones into lymphatic fluids, and the stimulatory action of urotensins I and II upon the lymph heart beat of the asiatic eel was investigated by Chan (1971). The lymph heart could assist the movement of urophysial secretions into the blood stream. Destruction by thermal cauterization of the lymph hearts of toads resulted in their death within four days (Zwemer and Foglia, 1943). These authors reported that,

"The failure of oedema fluid to return to the blood vessels in these experiments is a primary and uncomplicated cause of death".

The four anuran lymphatic hearts are large and the lymphatic spaces beneath the skin contain much more fluid than in eels (Thorson, 1964). Furthermore the multiple connections of the lymphatic vessels of the eel with the blood system provide alternative routes for fluid return.

Eels maintained in the laboratory occasionally contracted fungal and bacterial infections such as those described by Hine and Boustead (1974). Often the fungus was concentrated at the tip of the tail and resulted in the loss of the tip including the lymph heart. If treated with 'Furanace' or malachite green these infections usually cleared up leaving the fish without a functional lymph heart. These animals

survived in the laboratory for as long as fish that had not been infected. Loss of the lymph heart in the most acute manner was not fatal to the short-finned eel. For these reasons the function of the lymph heart was studied by the use of the isolated perfused tail preparation rather than by ablation of the heart.

II.8.2.1 General results

Lymph heart frequency and amplitude were recorded continuously in as many preparations as possible. In some cases no lymph heart beat record could be obtained and visual inspection revealed that it was inactive. Occasionally records could only be obtained when the tip of the transducer was pressed against the overlying skin at a force sufficient to produce a sustained depression in the body. This often resulted in a gradually diminishing amplitude and rendered the records unreadable. To alleviate this the transducer was withdrawn from its position between experiments to allow the lymph heart to recover. The anaesthetic used (Benzocaine) eliminated lymph heart muscle contraction. Thus records of the lymph heart were often absent or erratic for the first 15 to 25 minutes of perfusion. Records were often erratic when preparations were perfused without serum in the ringer. The size of the record was always enhanced by the addition of 5% human serum to the perfusate (see figure II.30).

After 20 to 40 minutes the lymph heart beat stabilized. Lymph heart records were seldom taken during tube perfusion. Where they were taken they were invariably of lower frequency and often erratic. This may indicate premature commencement of the Q/Pd profile experiments.

During the course of the experiments on each preparation there was no significant change in lymph heart frequency (LHf) as can be seen from table II.9. In this table the lymph heart frequency before the infusion of each concentration of AD is tabulated. Later it will be seen that AD administered in this manner increased both the frequency and amplitude of the lymph heart.

II.8.2.2 Response of the lymph heart to 5% human serum

Most preparations were either perfused with the basic saline or saline containing 5% human serum. In 14 preparations the saline was changed from the basic medium to that containing serum. The resistance response was biphasic. A small initial drop occurred at 1.6 ± 0.2 minutes, followed by a rise to reach a peak at 18.4 ± 3.2 minutes, (see figure II.30). This elevated resistance stabilised to a new baseline

FIGURE II.30 Typical record of the response of a perfused eel tail to addition of 5% human serum to the perfusate showing particularly the changes in lymph heart amplitude. Lymph heart frequency also increased after addition of serum to the perfusate.

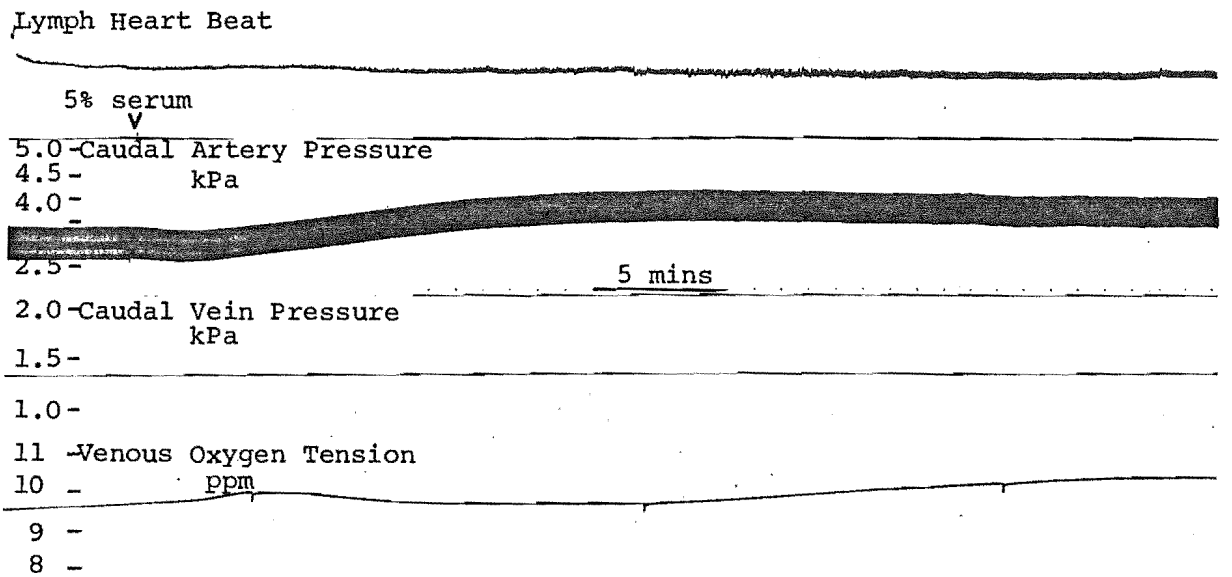

Lymph Heart Beat
5% serum
5.0
4.5
4.0
Caudal Artery Pressure
kPa
2.5
5 mins
2.0
Caudal Vein Pressure
kPa
1.5
1.0
11
10
Venous Oxygen Tension
ppm
9
8

Table II.9 Mean lymph heart frequency ± 1 S.E.M. (n = 8) immediately before the introduction of AD during the course of construction of cumulative dose response curves.

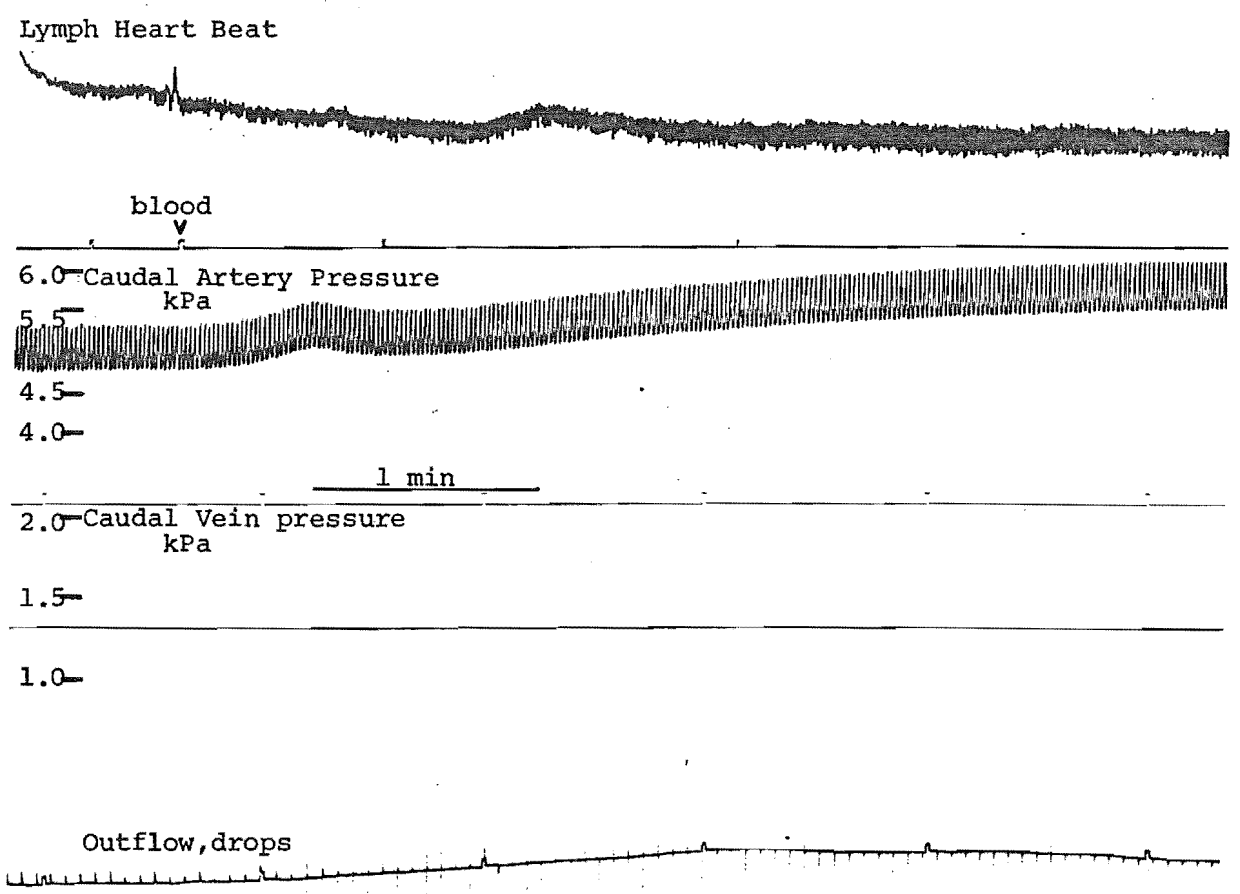
Concentration of AD	Mean lymph heart frequency ± 1 S.E.M.
1×10^{-8}M	62.12 ± 3.8
1×10^{-7}M	63.87 ± 4.0
1×10^{-6}M	63.0 ± 2.17
1×10^{-5}M	66.5 ± 4.07
1×10^{-4}M	68.2 ± 3.63
1×10^{-3}M	65.6 ± 7.06

over the following 30 minutes. The relatively long time course of this response and the need to operate the recorder at a high chart speed to obtain readable lymph heart records, (25-50 mm per minute) prevented continuous recordings of the responses in the interests of economy. Only one continuous record was taken from which the frequency can be read. In other preparations only occasional records were taken or the frequency was not readable leaving only amplitude.

Efficiency increased during the change to perfusion with serum ringer by 2.99 ± 0.38% which was not significant despite its occurrence in all but one of the fourteen experiments. Venous oxygen tension decreased by 0.26 ± 0.04 ppm (at 10 minutes, n = 4) during the rise in resistance but was consistently higher after perfusion with serum ringer, (+0.89 ± 0.08 ppm, at 30 minutes, n = 4). Lymph heart frequency and amplitude increased when the preparation was perfused with serum ringer, (see figure II.30). The amplitude change reached a stable level between 5 and 10 minutes. Frequency did not start to increase until after the first 10 minutes of perfusion with serum ringer. Initial lymph heart frequency for preparations perfused with the basic saline and saline containing 5% human serum were significantly different, ($p < 0.001$), the means being 53.54 ± 4.24 (n = 50) and 63.48 ± 5.14 (n = 30) respectively.

The mechanism of these responses is obscure. It could be suggested that the presence of serum proteins in the perfusate increases the force of contraction of the lymph heart beat by some non-specific means, (see section II.4.4, and Smith and Rozengurt, 1978). The frequency increase is likely to result from increased tissue fluid

FIGURE II.31 Typical record of response of a perfused eel tail to perfusion with whole eel blood. Lymph heart frequency and amplitude responses were developed more rapidly (< 120 s) than responses to addition of 5% human serum (see figure II.30).


Lymph Heart Beat
blood
6.0
Caudal Artery Pressure
kPa
5.5
4.5
4.0
1 min
2.0
Caudal Vein pressure
kPa
1.5
1.0
Outflow, drops

volume and pressure which would occur during the slow resistance rise. The frequency of the contractions in the presence of serum was more regular than in its absence. These features proved to be essential to accurately count the individual beats in the analysis of the records.

II.8.2.3 Response of the lymph heart to whole eel blood

Six preparations were perfused with whole eel blood previously collected from the fish during preparation for perfusion. Blood was stored for no more than five hours at $4^{\circ}C$ before being used. The small quantities collected allowed only brief periods of perfusion (4-6 minutes) but the response of the lymph heart was striking, (see figure II.31). Resistance increased by 21.05 ± 2.82% or 0.433 ± 0.108 kPa $ml^{-1}min$ after 56.2 ± 1.8 s (n = 6). Resistance then fell slightly to a plateau level that continued until the blood was replaced with saline. Amplitude of the lymph heart record increased to almost twice the initial values within the first two minutes and thereafter decreased. Lymph heart frequency rose by 5.54 ± 1.70 beats per minute within three minutes and remained higher than initial frequencies even after blood perfusion ceased, (see appendix A.37). Concomitant with the resistance rise was an increase in outflow which stablised at around three to four minutes. The outflow increase was maintained after blood perfusion ceased and was indicative of an increase in efficiency of ~ 6.62%, despite the fall in resistance which should decrease the outflow. The mechanism of this response is also unknown but the direction of the changes suggests that some factor or factors in human serum produce responses that are similar to those produced by blood perfusion.

II.8.3 Effects of AD, NAD and ISO upon lymph heart frequency and amplitude

Data on lymph heart frequency and amplitude changes are pooled from preparations perfused with and without serum in the ringer. Because of different initial lymph heart frequencies mean changes in frequency (ΔLHf) from the initial value were used for the analysis. The absolute size of the record in mm was used as a measure of amplitude.

II.8.3.1 Lymph heart and AD administered by constant infusion

At concentrations of less than 1×10^{-6}M AD no consistent changes in LHf or amplitude were observed. Higher concentrations produced increases in LHf and amplitude. Mean changes in LHf and amplitude with time during perfusion of AD at concentrations of 1×10^{-6}M AD and greater

are presented in figure II.32. Mean changes for all concentrations are tabulated in appendix A.38. Time taken for frequency and amplitude to peak or reach plateau levels is of the order of five to eight minutes after the start of perfusion. Amplitude of the records approximately doubled and peaked a minute or so before maximum frequency changes. Mean frequency increases of five to ten beats per minute were recorded for these higher concentrations.

II.8.3.2 Lymph heart and AD administered by bolus

Below 1 nmole AD no consistent changes in LHf or amplitude were observed. The greatest mean increase in LHf was 35 beats per minute and occurred nine minutes after injection of 10 nmoles AD. Five minutes after injection of 100 nmoles AD LHf increased by 15 beats per minute. This elevated frequency persisted for at least another ten minutes without further increase in the case of 100 nmoles AD, (see figure II.33 and appendix A.39).

II.8.3.3 Lymph heart and NAD

Insufficient records of LHf and amplitude during NAD infusion were available to construct graphs equivalent to those in figure II.34. Often only intermittent records were taken. Qualitatively however, LHf and amplitude increased when high concentrations of NAD were perfused through the preparations. Bolus administration of NAD produced results similar to those from bolus administered AD, (see figure II.34 and appendix A.40). The mean frequency increase for 100 nmoles NAD was ~ 10 beats per minute and is less than that for 10 and 100 nmoles of AD. These responses reached maxima at 5-10 minutes after injection.

II.8.3.4 Lymph heart and ISO, phentolamine and propranolol

Infusion or injection of ISO at concentrations or doses less than 1×10^{-4}M and 10 nmoles respectively produced small and unpredictable changes in lymph heart frequency and amplitude, despite extensive records. When large quantities of ISO were administered consistent small increases in LHf and amplitude comparable to those of AD and NAD were observed. The cause of these responses is believed to be the alpha constriction rather than beta stimulation which resulted from very high concentrations and doses of ISO.

Phentolamine and propranolol both reduced lymph heart amplitude, but had no effect on lymph heart frequency. Amplitude increases in response to alpha agonists occurred slightly before frequency responses

FIGURE II.32 Changes in lymph heart frequency and amplitude during infusion of adrenaline at concentrations between 1×10^{-8}M and 1×10^{-3}M. Mean initial lymph heart frequency and mean changes in lymph heart frequency and amplitude are tabulated for all concentrations in appendix A.38. Amplitude changes occurred before frequency changes.

a. Changes in lymph heart frequency during infusion of 1×10^{-8}M adrenaline.

b. Changes in lymph heart frequency during infusion of 1×10^{-7}M adrenaline.

c. Changes in lymph heart frequency during infusion of 1×10^{-6}M adrenaline.

d. Changes in lymph heart amplitude during infusion of 1×10^{-6}M adrenaline.

e. Changes in lymph heart frequency during infusion of 1×10^{-5}M adrenaline.

f. Changes in lymph heart amplitude during infusion of 1×10^{-5}M adrenaline.

g. Changes in lymph heart frequency during infusion of 1×10^{-4}M adrenaline. Histogram on abcissa indicates the number of preparations 'swimming' (see right hand ordinate).

h. Changes in lymph heart amplitude during infusion of 1×10^{-4}M adrenaline. Histogram on abcissa indiates the number of preparations 'swimming' (see right hand ordinate).

i. Changes in lymph heart frequency during infusion of 1×10^{-3}M adrenaline. Histogram on abcissa indicates the number of preparations 'swimming' (see right hand ordinate). Because of the 'swimming' response it was not possible to measure lymph heart amplitude during infusion of 1×10^{-3}M adrenaline.

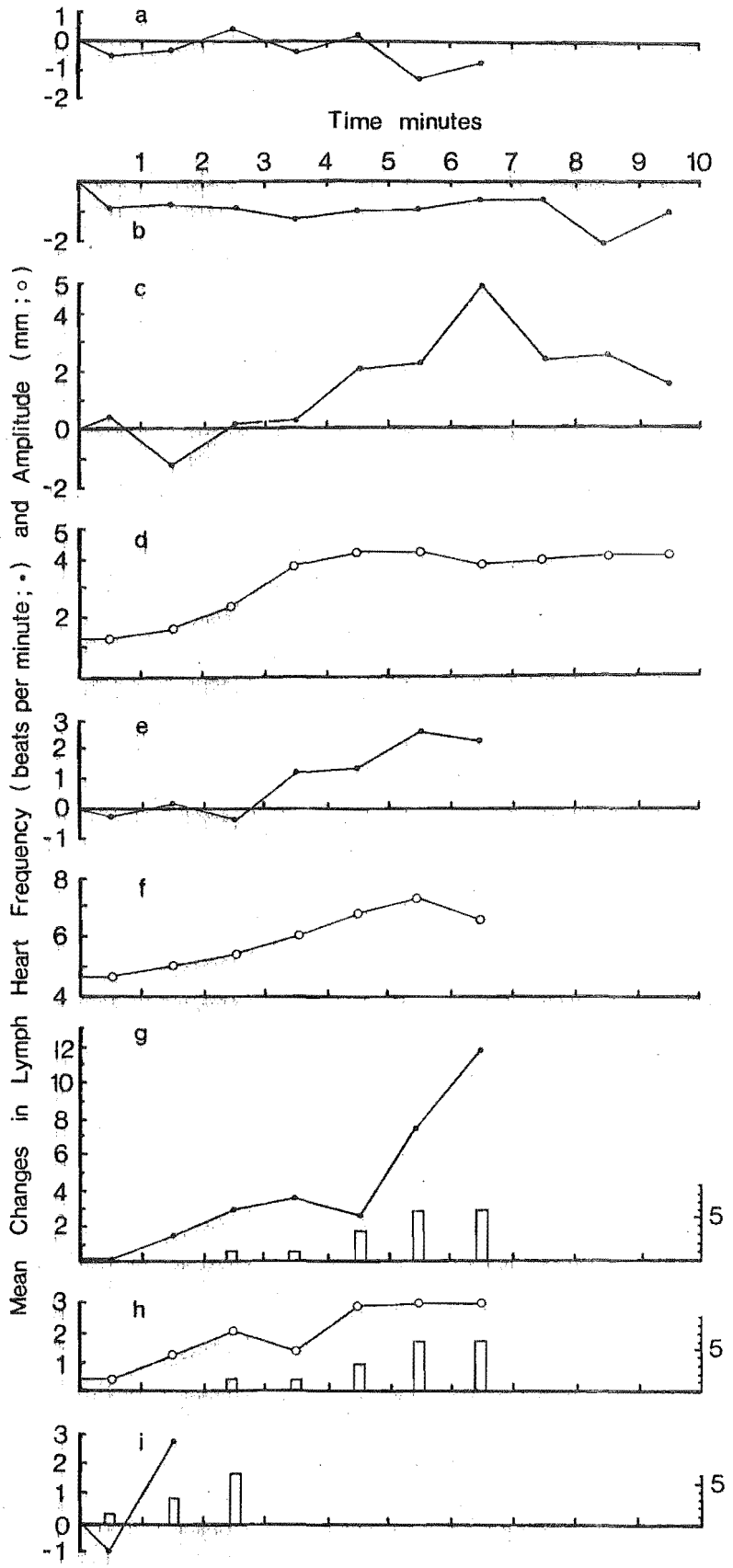

Time minutes
1 2 3 4 5 6 7 8 9 10
a
b
c
d
e
f
g
h
i
Mean Changes in Lymph Heart Frequency (beats per minute ; •) and Amplitude (mm ; ○)

FIGURE II.33 Changes in lymph heart frequency with time after injection of 1, 10 and 100 nmoles adrenaline. Mean initial lymph heart frequency and mean changes in lymph heart frequency for these doses are tabulated in appendix A.39.

a. Changes in lymph heart frequency with time after injection of 1 nmole adrenaline. Lymph heart frequency changed little in response to doses less than 1 nmole. Note that ordinate scale is five times that of the following two graphs, b,c.

b. Changes in lymph heart frequency with time after injection of 10 nmoles adrenaline.

c. Changes in lymph heart frequency with time after injection of 100 nmoles adrenaline.

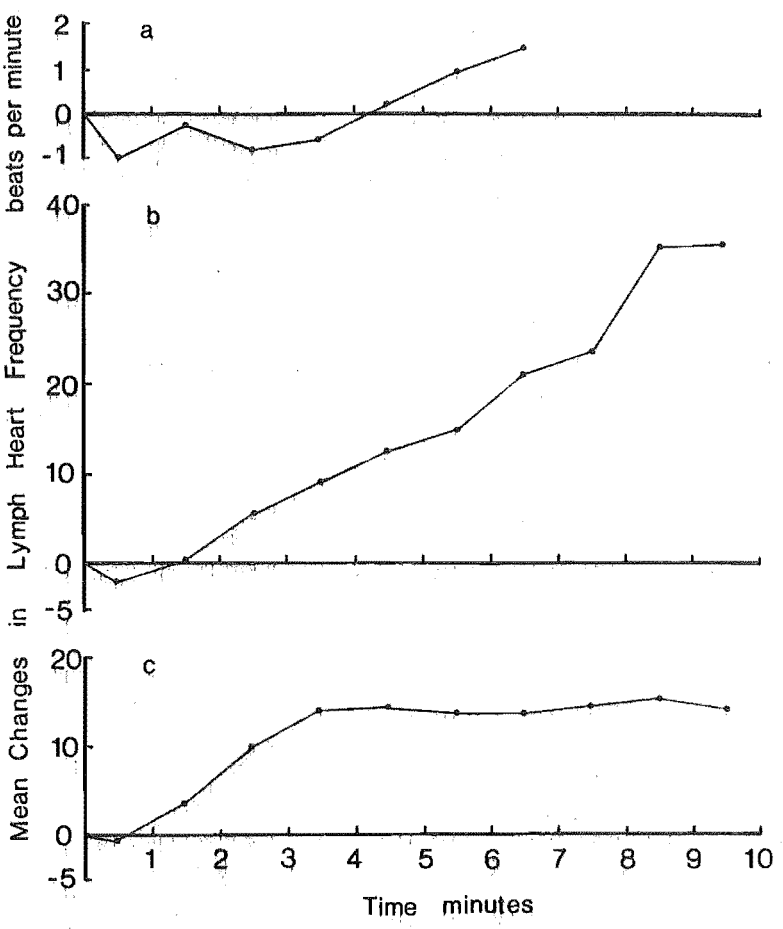
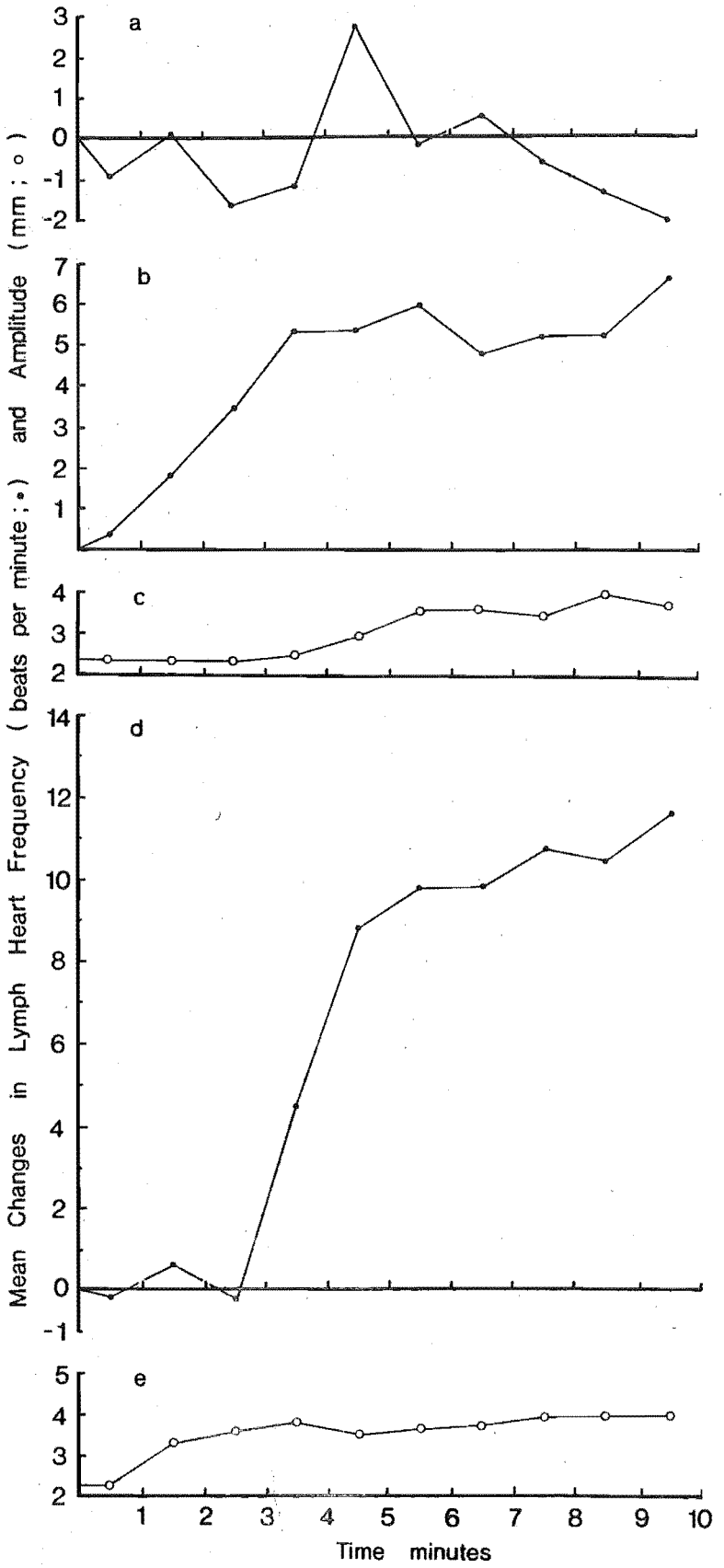

Mean Changes in Lymph Heart Frequency beats per minute
a
b
c
2
1
0
-1
40
30
20
10
0
-5
20
10
0
-5
1
2
3
4
5
6
7
8
9
10
Time minutes

FIGURE II.34 Changes in lymph heart frequency and amplitude with time after injection of 1, 10 and 100 nmoles of noradrenaline. Mean initial lymph heart frequency and amplitude and mean changes in frequency and amplitude for these doses and 500 pmoles are tabulated in appendix A.40.

a. Changes in lymph heart frequency with time after injection of 1 nmole noradrenaline. Lymph heart frequency and amplitude changed little in response to doses less than 1 nmole, (see appendix A.40).
b. Changes in lymph heart frequency with time after injection of 10 nmoles noradrenaline.
c. Changes in lymph heart amplitude with time after injection of 10 nmoles noradrenaline.
d. Changes in lymph heart frequency with time after injection of 100 nmoles noradrenaline.
e. Changes in lymph heart amplitude with time after injection of 100 nmoles noradrenaline.


a
b
c
d
e
Mean Changes in Lymph Heart Frequency (beats per minute ; •) and Amplitude (mm ; ○)
Time minutes

(see figures II.32-II.34), and when combined with the observations of Davie, (1975; see section I.4.3) indicate positive ionotropic responses resulting from adrenergic stimulation of some part of the control system of the lymph heart. Reduction of lymph heart amplitude by phentolamine and propranolol may be due to alpha blockades since evidence of partial alpha antagonistic activity by propranolol has been produced, (see section II.6.3.4). That ISO has no effects upon lymph heart frequency or amplitude indicates that propranolol induced amplitude depression is not the result of beta blockade. As the alpha antagonistic potential of phentolamine is greater than propranolol a larger drop in lymph heart amplitude in response to phentolamine could be expected. In fact from the few complete sets of records available, propranolol reduces lymph heart amplitude relatively more than phentolamine. A possible explanation of lymph heart amplitude depression by propranolol is provided by Larsen and Teravainen (1978) who reported that d- and l-propranolol have curare like effects upon cholinergic nerve terminals. Since lymph heart muscle appears to be excited by cholinergic nerves this may be significant (see section I.6.1). Larsen and Teravainen (1978) reported that propranolol reduces only the size of the response and leaves the time course and resting muscle potential unaffected.

Clearly there is still much to be learnt about the pharmacology of the lymph heart, and since amplitude records of lymph heart beat in this preparation are rather unreliable, further discussion is unwarranted.

II.8.4 Discussion of the function of the lymph heart

Measurement of the outflow rate changes (during administration of drugs) allows estimation of changes in vascular and extravascular volumes. The two components can be separated by their different time courses and directions during vasoconstriction. Vascular volume adjustments occur rapidly (< 120 s), and are decreases. They occur simultaneously with resistance changes. Extravascular volume increases when AD and NAD are given, and peaks at three to five minutes after drug introduction, (see section II.7.5). Increases in extravascular fluid could be expected to increase lymph heart output since this is one route for the return of tissue fluid to the vascular system. However lymph heart activity could be directly influenced by the drugs AD and NAD.

If AD or NAD directly affects lymph heart activity then the response could be expected at or near the time that the drug contacts the lymph heart tissue. The rapid perfusion of the tail vessels, including

those of the lymph heart muscles (~ 90 s, see section II.5.4), and the short time taken for the resistance vessels to reach maximal constriction, (60-90 s) clearly shows that the drugs reach the lymph heart tissue within two minutes. Maximal lymph heart frequency increases occur at seven to nine minutes after the introduction of high doses of AD administered by bolus or constant infusion. Likewise frequency reaches maxima five to ten minutes after injection of 1, 10 or 100 nmoles NAD, (see table II.10). Chan (1971) recorded lymph heart frequency after injection of approximately 30 nmoles of either AD or NAD into asiatic eels. A peak increase in LHf of 23 beats per minute was reported after 25 minutes. The time lag between contact of

Table II.10 Time (minutes) taken to reach peak resistance, outflow and lymph heart changes in response to AD and NAD.

Adrenaline (perfusion line)

	1×10^{-6}M	1×10^{-5}M	1×10^{-4}M	1×10^{-3}M
Resistance	2.33	2.16	1.56	1.24
ΔOQ	5.0	5.0	5.0	3.0
ΔLHf	7.0	6.0	8.0	-

Adrenaline (bolus)

	1 nmole	10 nmoles	100 nmoles
Resistance	0.975	1.18	1.13
ΔOQ	3.0	3.5	4.0
ΔLHf	7.0	9.0	5.0

Noradrenaline (bolus)

	1 nmole	10 moles	100 nmoles
Resistance	1.61	1.54	1.74
ΔOQ	3.4	3.0	4.0
ΔLHf	5.0	6.0	10.0

the drug and the lymph heart tissue and the activity change of the lymph heart, needs some explanation. When eel blood was perfused through the preparation, LHf and amplitude increased within two minutes, (see section II.8.2.3). Thus natural blood borne stimulators, (whatever their nature) are capable of inducing rapid increases in frequency and amplitude. This rapid type of response was not observed when AD and NAD were given. Amplitude changes reported here occurred before frequency changes. Thus amplitude may be controlled by a different mechanism to frequency, perhaps by stimulation of the Na^+/K^+ pump of the muscle cells (see Akasu, Ohta and Koketsu, 1978). The recorded changes in frequency appear to be the result of other than direct action of AD and NAD. Increases in frequency of the lymph heart of the asiatic eel in response to 5-HT were rapid and dose dependent (Chan, 1971). Although Chan suggested that the action of 5-HT was likely to be upon some part of the neural control circuit of the lymph heart, it is possible that 5-HT induced capillary permeability increases (Myhre, Steen and Stray-Pedersen, 1976) may have been partly responsible for the frequency response.

Increases in extravascular fluid volume and pressure appear to be logical causes for LHf increases in response to AD and NAD. Lymph heart frequency increases occurred only at high concentrations or doses of AD and NAD. Likewise significant changes in extravascular fluid also occurred only under these conditions. Table II.10 shows that the peak increase in extravascular volume occurred before the peak lymph heart frequency changes under all conditions. Should lymph heart frequency have increased before extravascular volume then the hypothesis would have had to be rejected. To look at the relationship between lymph heart frequency and extravascular volume the hypothesis that AD and NAD caused increased extravascular fluid build up and that this subsequently elevated lymph heart frequency was formed. To test this hypothesis the cumulative decreases in outflow after the initial vascular volume adjustment, were calculated at each minute and are taken to reflect progressive increases in extravascular fluid volume. Outflow changes (ΔOQ) were then plotted against mean changes in lymph heart frequency, (ΔLHf). A model II type regression, where the independent variable is measured with significant error, was used to analyse the data and determine if a significant relationship existed between ΔOQ and ΔLHf, (Simpson, Roe and Lewontin, 1960).

The graph of mean ΔLHf versus mean cumulative ΔOQ for each minute of perfusion with AD at concentrations of 1×10^{-6}M AD and greater is

presented in figure II.35. There is a significant relationship ($P < 0.05$) between the two variables which is described by the equation with the figure. When the data were not pooled, only 1 x 10^{-5}M AD revealed a significant relationship, ($P < 0.05$). Likewise the mean ΔLHf and mean cumulative ΔOQ for each minute after injection of 1, 10 and 100 nmoles AD were treated in the same manner. There is a significant relationship between the two variables, ($P < 0.05$, see figure II.36). At each individual dose only 10 nmoles AD produced a significant relationship, ($P < 0.05$). The data from 100 nmoles AD are significantly related when the natural logs of each of the variables are taken, ($P < 0.01$).

For bolus administration of 1, 10, 100 nmoles NAD the pooled data yield a significant linear relationship between the changes in lymph heart frequency and cumulative changes in outflow rate, ($P < 0.05$, see figure II.37). At none of the individual doses is the relationship significant.

The correlation coefficient for ΔLHf versus ΔOQ for all data from AD administered by the perfusion line and as a bolus, and NAD as a bolus was computed and is equal to 0.399 which is a significant correlation at $p < 0.01$, for 77 data point pairs. The regression equation for all pooled data is

$$\Delta LHf = -19.23 \ \Delta OQ + 0.1549$$

The slopes of the regression lines are not significantly different for the data from infused AD and injected AD. This suggests that the change in lymph heart frequency per unit change in extravascular volume is not dependent upon the method of administration of the drug.

At this point it is interesting to do some arithmetic on the volumes of fluid transported by the lymph heart. From the dimensions of the lymph heart (see section I.4.3) the volume of the lymph heart when distended has been estimated by the volume displacement of a scale model to be between 0.16 µl and 0.35 µl. At an average resting frequency of 58 beats per minute this would result in the replacement of 9-20 µl of lymph into the caudal vein per minute. This calculation assumes that all the fluid contained in the heart is expelled. In an average sized fish of say 400 g the extravascular extracellular fluid volume of the tail would be about 20 ml. Thus the lymph heart could pump the entire tail interstitial volume in 16 to 37 hours, which is comparable to the times for lymph circulation in mammals (Mayerson, 1962).

FIGURE II.35 Mean changes in lymph heart frequency versus mean cumulative changes in venous outflow during infusion of adrenaline at concentrations between 1×10^{-6}M and 1×10^{-3}M. Linear model two type regression (Bartlett's 'best fit' method where both variables are measured with significant error) gave the fitted line which has the equation as follows,

$$\Delta LHf = -18.5\Delta OQ - 0.0332$$

Student's t-test value for the significance of the single regression was = 2.977, for 7 d.f., which is significant at $p < 0.05$ ($t_{0.05\ [7]} = 2.365$)

△ 1×10^{-6}M AD

■ 1×10^{-5}M AD

▲ 1×10^{-4}M AD

□ 1×10^{-3}M AD

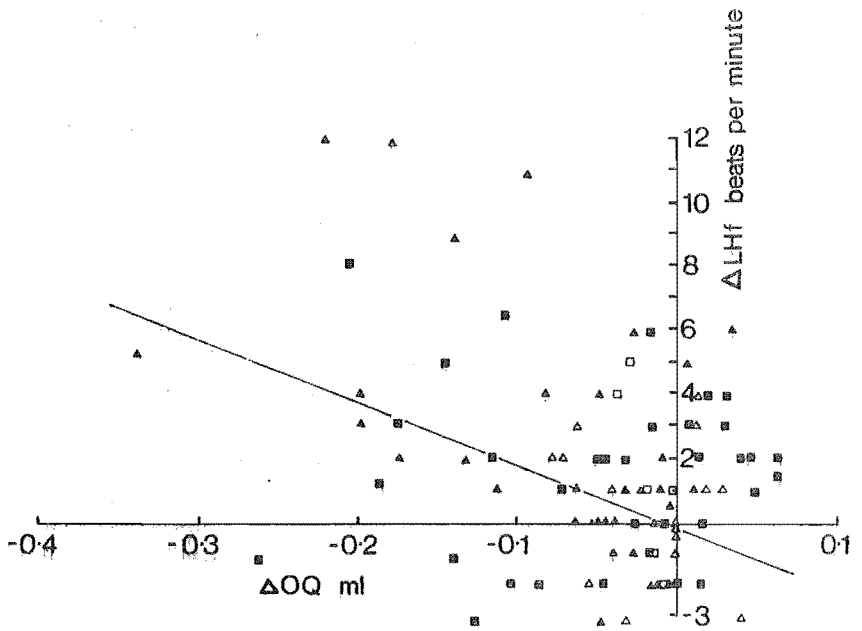

ΔLHf beats per minute
12
10
8
6
4
2
-3
-0·4
-0·3
-0·2
-0·1
0·1
ΔOQ ml

FIGURE II.36 Mean changes in lymph heart frequency versus mean cumulative changes in venous outflow after injection of adrenaline at doses of 1, 10 and 100 nmoles. Linear model two type regression (Bartlett's 'best fit' method where both variables are measured with significant error) gave the fitted line which has the following equation,

$$\Delta LHf = -21.9\Delta OQ + 6.38$$

Student's t-test value for the significance of the single regression was = 3.329, for 7 d.f., which is significant at $p < 0.05$. ($t_{0.05\,[7]} = 2.365$; $t_{0.01[7]} = 3.499$).

△ 1 nmole AD

■ 10 nmoles AD

▲ 100 nmoles AD

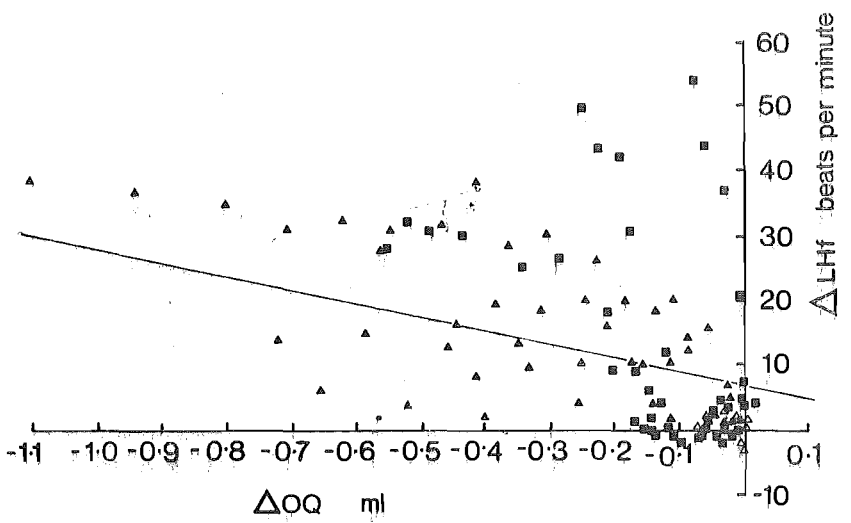

60
50
40
30
20
10
-10
ΔLHf beats per minute
-1·1 -1·0 -0·9 -0·8 -0·7 -0·6 -0·5 -0·4 -0·3 -0·2 -0·1 0·1
ΔOQ ml

FIGURE II.37 Mean changes in lymph heart frequency versus mean cumulative changes in venous outflow after injection of 1, 10 and 100 nmoles noradrenaline. Linear model two type regression (Bartlett's 'best fit' method where both variables are measured with significant error gave the fitted line which has the following equation,

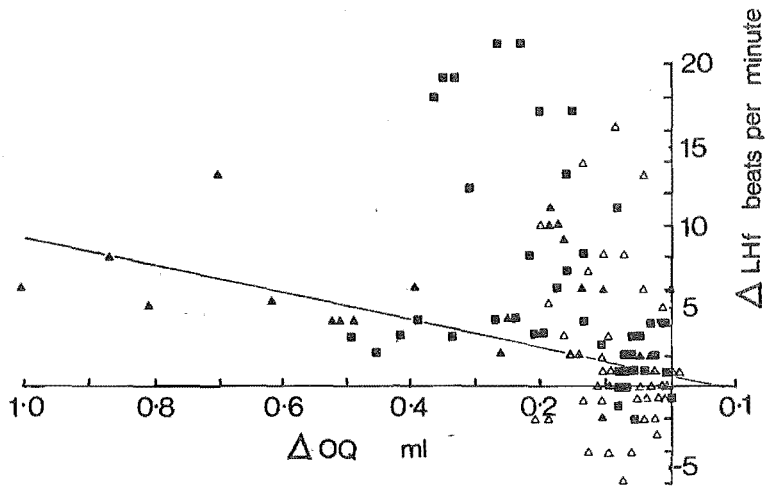
$$\Delta LHf = -8.09\Delta OQ + 0.958$$

Student's t-test for the significance of the single regression was = 7.957 for 9 d.f., which is significant at $P < 0.001$ level. ($t_{0.001\,[9]} = 4.781$).

△ 1 nmole NAD

■ 10 nmoles NAD

▲ 100 nmoles NAD


20
15
10
5
-5
Δ LHf beats per minute
1·0
0·8
0·6
0·4
0·2
0·1
Δ OQ ml

The lymph heart responses that occurred when venous outflow decreased are significantly correlated. We can calculate the amount of extravascular fluid replaced after extravasation of a set amount of perfusate. For example, for each 0.1 ml of fluid lost to the tissue, lymph heart frequency increased by approximately two beats per minute. From the above figures, ~ 0.7 µl of fluid would be replaced into the blood stream for every 100 µl that are lost. Despite the rather gross assumptions about the volumes pumped by the lymph heart it is clear that the lymph heart would have to maintain elevated frequency and/or amplitude for a long time to significantly reduce the excess extravascular fluid produced in the conditions described in these experiments. In the absence of muscular contractions which would increase lymph flow to the lymph heart, these results are probably conservative and reflect a volume buffering capacity of the interstitium and lymph vessels (Jacobson and Kjellmer, 1964; Gnepp and Sloop, 1978).

I believe however that the experiments described here show that one of the functions of the lymph heart is to assist in removal of extravascular fluid in the face of impending oedema, and replace this fluid into the blood stream. The mechanism by which lymph heart frequency and perhaps amplitude increased in response to extravasation is unclear, although an hypothesis can be formed, and the chain of events could be envisioned as follows. Adrenaline or noradrenaline, when introduced into the preparation cause vasoconstriction thereby increasing the capillary pressure and permeability resulting in extravasation of perfusate. Build up of extravascular fluid volume and pressure results in greater lymphatic flow toward the lymph heart driven by higher extravascular and lymphatic fluid pressures. This flow would persist after the removal of AD or NAD because the extravascular fluid volume may increase in response to these drugs. Greater volumes of lymph reaching the lymph heart at higher pressures will increase distension of the lymph heart when filling. Lymph heart frequency is controlled by the frequency of the pulses of motor impulses transmitted from the spinal cord above the rostrally adjacent vertebra to the lymph heart muscle via the lymph heart nerve, (see section I.5). When the lymph heart muscles are mechanically distended sensory spikes are observed in the lymph heart nerve. Thus by a simple neural feedback loop the frequency of the lymph heart could be modulated by changes in extravascular fluid volume.

II.9 Summary

1. The arterial and venous blood system of the short-finned eel tail is described with detail of the blood and lymphatic vasculature in the seven most posterior vertebral segments of the tail which include the caudal lymphatic heart (section II.2).

2. An isolated saline perfused eel tail preparation is described which took approximately 30 minutes to prepare (section II.3). Vascular resistance of this preparation was relatively constant during experiments and was free from gradually increasing baseline resistance with time, indicative of deterioration of the vascular bed. Approximately 87% of the perfusate pumped into the preparation emerged from the venous cannula. All tail vessels were well perfused with a circulation time through the tail of 60-90 s (section II.5.5). Perfusion by either the vertical tube method or by peristaltic pump gave estimates of vascular resistance that were not significantly different (section II.5.4).

4. Mean baseline resistances from 86 perfused tails at caudal venous pressures of zero and 1.33 kPa, with and without serum added to the perfusate are given in table II.1. These values agree well with the few *in vivo* measurements of trans-tail pressure differentials. Baseline resistance was inversely proportional to the weight of the tissue perfused. Increased venous pressure decreased resistance by approximately 10% per 0.5 kPa increment.

5. Addition of small amounts (3% or 5% vol./vol.) of human serum to the perfusate significantly increased baseline resistance. This increase in vascular tone was principally due to the high molecular weight (> 12000) molecules (section II.5.3). Serum in the perfusate conferred a degree of autoregulation of resistance to the preparations and increased responsiveness to catecholamines. Although serum in the perfusate increased responsiveness to catecholamines, it had little effect upon estimates of pharmacological parameters such as ED50 (section II.6.3.1).

6. Dose versus resistance response curves for adrenaline, noradrenaline and isoprenaline, either infused at constant concentrations in the perfusate or injected as 0.1 ml boli are presented. Summaries of data from these curves are presented in tables II.3 (infusion) and II.4 (injection). Administration method did not appear to alter the response magnitude to the same molar quantity of drug (section II.6.3.3).

7. Adrenaline and noradrenaline caused increased tail vascular

resistance (vasoconstriction) at all concentrations and doses tested. Isoprenaline caused decreased resistance (vasodilation) at concentrations below 1 x 10^{-4}M and at all injected doses tested. Concentrations of 1 x 10^{-4}M and 1 x 10^{-5}M isoprenaline, increased resistance.

8. Adrenaline was 1.14 to 4.31 times more potent a vasoconstrictory drug than noradrenaline (section II.6.3.2).

9. On the basis of the potency comparisons of these three drugs and additional evidence from the use of selective adrenergic antagonists (section II.6.4) the eel tail vascular bed was found to contain alpha constrictory and beta two dilatory adrenergic receptors.

10. Responses of the eel tail to exogenous catecholamines suggest that control of vascular resistance in the eel tail is of a dual nature. Circulating catecholamines elevate resistance and sympathetic nervous activity probably decreases resistance (section II.6.6).

11. The vascular volume of the perfused eel tail preparation was approximately 3.1 ml per 100 g tail weight (section II.7.2). The decrease in tail vascular volume during vasoconstriction was less than 2% of the eel tail vascular volume for any concentration or dose of adrenaline or noradrenaline. Increases in vascular volume during isoprenaline mediated vasodilation were less than 1%. The size of the changes in vascular volume is explicable in terms of the changes in volume of the precapillary resistance vessels (section II.7.4).

12. Mean increases in interstitial volume during infusion of adrenaline at concentrations greater than 1 x 10^{-6}M were less than 3% of the estimated interstitial volume of the perfused eel tail preparation. Injection of doses of adrenaline and noradrenaline greater than 1 nmole caused mean increases of less than 11% of the estimated interstitial volume. Isoprenaline caused small and inconsistent changes in interstitial volume at concentrations below 1 x 10^{-4}M (section II.7.5). Changes in interstitial volume appear to be the result of extravasation of perfusate at high luminal pressures during vasoconstriction.

13. Lymph heart frequency was reliably and accurately recorded in this preparation by a non-invasive technique. Because of the nature of the recording apparatus, lymph heart amplitude was recorded with limited accuracy. Mean initial lymph heart frequency was 53.5 and 63.5 beats per minute when the preparations were perfused without and with serum added to the perfusate respectively. Lymph heart frequency did not change significantly throughout the life of a preparation between experimental manipulations. Lymph heart frequency and amplitude increased in response to 5% serum in the perfusate (section II.8.2.2).

Lymph heart frequency and amplitude increased when whole eel blood was infused into the preparation (section II.8.2.3).

14. Lymph heart frequency and amplitude increased when high concentrations (> 1 x 10^{-6}M) and doses (> 1 nmole) of adrenaline and noradrenaline were administered. Isoprenaline at concentrations which caused dilation elicited no response from the lymph heart. At high concentrations (> 1 x 10^{-4}M) slight increases in lymph heart frequency and amplitude were recorded. Adrenergic antagonists had little effect on lymph heart frequency although amplitude was depressed. Lymph heart amplitude may be increased by alpha adrenergic stimulation (section II.8.3).

15. Lymph heart frequency increases were significantly correlated with increases in interstitial fluid volume. One function of the lymph heart is to assist in recovery of extravasated fluid.

PART III CARDIOVASCULAR RESPONSES TO SWIMMING IN THE SHORT-FINNED EEL

III.1 Introduction

The study of circulatory responses to exercise in teleosts has led to significant contributions to our understanding of teleost cardiovascular homeostasis. Information from isolated perfused vascular preparations (see Wood, 1974a, 1975) and changes in plasma catecholamine concentrations during stress (see Mazeaud et al., 1977) have supported the hypothesis that resistance to blood flow in various vascular beds is controlled by circulating catecholamines (see Randall and Stevens, 1967; Burnstock, 1969). The ability of this hypothesis to explain cardiovascular responses to exercise is briefly considered here.

Two sets of experiments have been reported where cardiac output and pressures in ventral and dorsal aortas have been measured in rainbow trout during swimming by Randall and Stevens (1967), Stevens and Randall (1967a,b) and more recently by Kiceniuk and Jones (1977). Both of these studies revealed increases in cardiac output and decreases in branchial and systemic resistances during swimming. Cardiac output increases of 2-4.5 fold are sufficient to account for the fall in branchial resistance in terms of passive distensibility, provided that there was no vascular tone already present which could limit distension (Folkow and Lofving, 1957). The large number of reports of decreased branchial resistance in response to exogenous catecholamines in both isolated gills (Wood, 1974b) and holobranchs (Rankin and Maetz, 1971) and *in vivo* (Randall and Stevens, 1967) indicate that there is resting vascular tone in the branchial vessels. Isolated gill and holobranch preparations give resting values of branchial resistance that are similar to, or greater than, measurements of branchial resistance *in vivo*. Thus resting vascular tone in the gills seems not to be maintained by circulating catecholamines or by sympathetic nervous activity. Perhaps the structure of the gill vessels is such that it provides the resting vascular tone upon which dilatory responses are superimposed. The predominant response of the gills to catecholamines is beta one mediated adrenergic dilation, thus it has been proposed that elevated blood catecholamine concentrations caused the fall in branchial resistance during exercise.

Swimming in trout is associated with a rise in dorsal aortic and venous pressures. Dorsal aortic venous pressure differentials

when divided by the proportionally greater blood flow rates during exercise indicate falls in systemic resistance. Although slow release of lactate from muscles in exercising fish may indicate low blood flow rates within skeletal muscle (Black et al, 1959; Driedsic and Kiceniuk, 1976), Stevens (1968) found no significant differences in blood distribution with exercise in rainbow trout, thus we have no evidence to suggest redirection of blood away from the swimming muscles during swimming. In this species, as in mammals (Folkow, 1960), systemic vascular tone is probably maintained by sympathetic nervous activity (Wood, 1974b; Smith, 1978). Since the predominant response of the systemic vasculature to catecholamines is alpha mediated constriction, (Wood and Shelton, 1975) elevated catecholamine concentrations are unable to explain the falls in systemic resistance with exercise reported by Randall and Stevens (1967), Stevens and Randall (1967a,b) and Kiceniuk and Jones (1977). The hypothesis outlined in the first paragraph clearly needs re-examination. The systemic resistance decreases could be due to a number of mechanisms such as decreased sympathetic tone, passive distensibility at elevated luminal pressures or local dilation caused by metabolites.

Salmonids should be good subjects for exercise physiology studies in fishes because of their naturally active behaviour. Stevens, Bennion, Randall and Shelton (1972) provided data from the lingcod, (*Ophiodon elongatus*), during brief swimming activity. Cardiac output fell during swimming, the cause of which was suggested to be vagal inhibition of heart activity. This response was similar to a 'disturbance' reaction and may not reflect a response to exercise. Blockade of vagal inhibition by atropine resulted in 1.25 to 2 fold increases in cardiac output during swimming activity. Associated with swimming were decreases in branchial resistance and increases in peripheral resistance. It is unfortunate that these authors had no means of inducing sustained swimming in their fishes. The initial bradycardia may have been overcome as the metabolic demands of exercise increased with time. The results from unatropinised lingcod are in contrast with those from trout but are hardly comparable.

Johansen, Franklin and Van Citters (1966) reported that blood velocity in the ventral aortas of various elasmobranch species changed little during periods of exercise of up to 30 minutes. They also noted that after swimming there was an increase in ventral aortic blood flow.

The results from lingcod and elasmobranchs suggest a different pattern of responses from those observed in trout, but the limited data

available provides only tentative answers as to how these responses were invoked. Clearly more species need to be studied to establish the array of events that actually occur.

At times of high oxygen demand, changes in the pattern of blood flow through the gills so as to increase gas exchange have been suggested (Hughes and Morgan, 1973). These changes might be reflected as changes in branchial resistance. In this section short-finned eels were swum at two speeds for periods of 30 minutes or more while cardiac output, ventral aortic pressures, dorsal aortic pressures and caudal venous pressures were recorded. These data should indicate any changes in resistance across the gills and tail vascular beds and provide a suitable comparison for the data from Randall and Stevens (1967), Stevens and Randall (1967a,b) and Kiceniuk and Jones (1977). They should also provide information on the possible participation of plasma catecholamines in the observed responses.

The experiments described in this section (III) are the result of combined work by Dr M.E. Forster and P.S. Davie with technical assistance from Ms H.J. Sterritt. The nature of the project particularly the surgery, required the combined efforts of all three participants. In so far as the design and interpretation of experiments is concerned, equal contributions were made by Dr M.E. Forster and P.S. Davie.

III.2 Materials and Methods

III.2.1 Test apparatus

The experimental tank was similar to that described by Priede (1974), (see figure III.1). Oxygen tension was determined with a Beckman Field Lab Oxygen Analyser with 3995 O_2 sensor and found to be 95-100% air saturated. The upstream half of the experimental chamber was covered with a black cloth to eliminate visual disturbances to the fish.

Each fish was placed in the experimental chamber and forced to swim against a current for a period of at least three hours, on the day prior to surgery. At this time the stainless steel grids were electrified and mild shocks administered if the fish failed to maintain station.

Measurements of water temperatures of the river at collection and of the holding tanks were between 15.5°C and 17.0°C. Water in the flume was maintained at mean temperature of 16.78 ± 0.17°C.

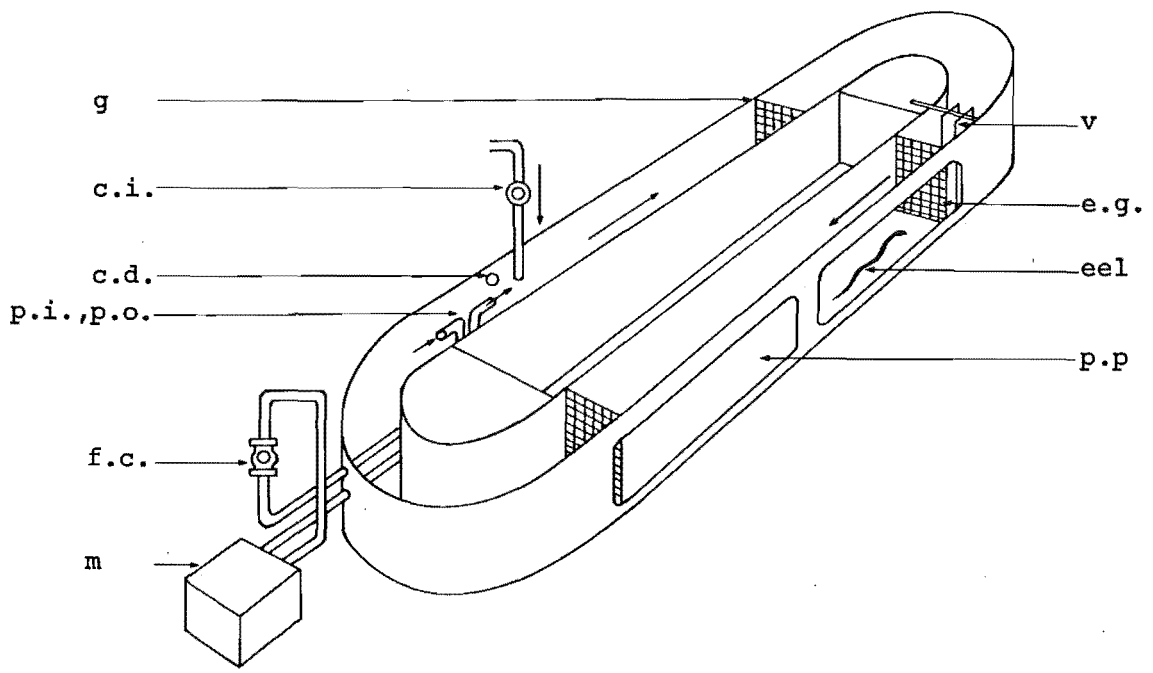
III.2.2 Surgical procedures

Eels were anaesthetised in a 0.04% solution of benzocaine in water. Throughout surgery the gills were irrigated with an aerated solution of anaesthetic (0.008%), pumped into the buccal cavity by a peristaltic pump (Cole Parmer, Masterflex). After surgery the gills were irrigated with tap water until opercular movements started.

The ventral aorta, dorsal aorta and caudal vein were cannulated with the fish in a supine position and the three cannulae were securely anchored to the body wall and were tied together at intervals to minimise tangling. The caudal vein was cannulated via one of its branches which was dissected free of the connective tissue sheath of the right-hand side of the kidney, just caudal of the vent. The free end of the cannula lay in the caudal vein, about 0.5 cm cephalad of the first haemal arch, and pointing upstream. The dorsal aortic cannula was tied into the gonadal artery after that vessel was dissected free of the overlying (i.e. ventral) pneumogastric artery. The free end of the cannula lay in the lumen of the dorsal aorta, pointing upstream. The ventral aortic flow probe and cannula was tied into the ventral aorta between the bulbus arteriosus and first afferent branchial arteries, effectively replacing c. 1.5 cm of the blood vessel. The ventral aorta was exposed by a ventral incision through the

FIGURE III.1 Diagram of flume for swimming fish. The straight channel was 140 cm long, 20 cm deep and 15 cm wide and was bounded at both ends by stainless steel grids which could be connected to a 45 V A.C. source.

Water was forced around the flume by a 1 h.p. Davies S.P. pump. Water speeds were measured with a Kent Lea Mini Flo Probe and could be varied by a valve on the pump outlet between 0 and 50 cm s. Two vanes at the upstream end of the experimental chamber were adjusted to minimise turbulence and remove flat spots through the chamber. Water was constantly replaced by tap water from an artesian supply. c.d, constant level drain; c.i, constant temperature water inflow; e.g, electric grid; f.c, flow control valve; g, grid to smoothen flow; m, motor and pump; p.i, and p.o, pump inflow and outflow; p.p, perspex panel for observation; v, vanes to direct flow.


g
c.i.
c.d.
p.i.,p.o.
f.c.
m
v
e.g.
eel
p.p

mesentery separating the two sets of opercular muscles. Strong finger pressure applied to the heart, through the body wall, at the time that the incisions were made in the ventral aorta, prevented blood loss from the aorta. Thus blood loss was limited to that volume held in the ventral aorta. It was also necessary to clamp the downstream end of the ventral aorta to prevent backflow of blood.

The cannulae were filled with a fresh water eel saline (Rankin and Maetz, 1971) to which was added heparin (100 i.u. ml^{-1}, ammonium salt, Sigma Chemical Co.). After surgery the fish were injected via the caudal vein cannula with 1 mg heparin in 0.2 ml Ringer's solution at 12 hr intervals. While not recording the cannulae were plugged with short lengths of wire.

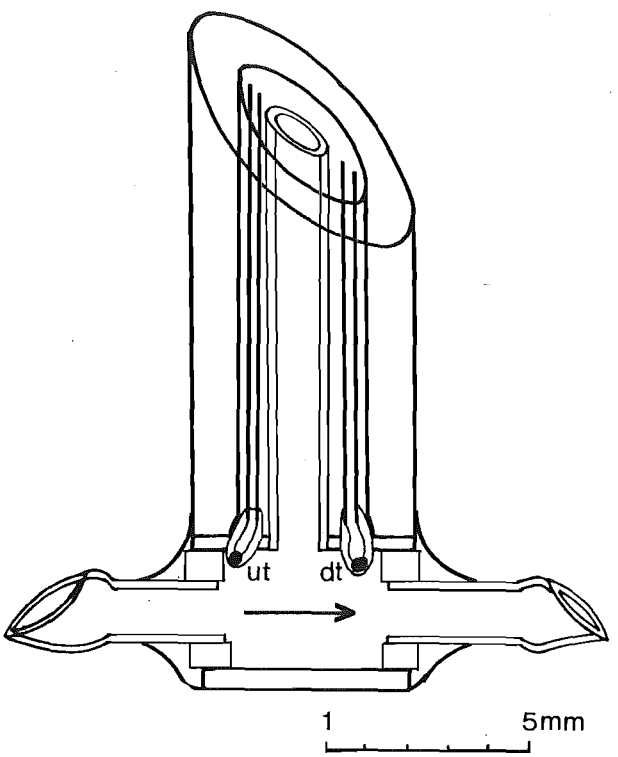
The fish were placed in the flume and their condition checked at frequent intervals to minimise tangling of the cannulae. During recovery and at rest a low rate of flow (< 5 cm sec^{-1}) was maintained in the experimental chamber.

III.2.3 The flow probe

The principle of the thermal flow probe is as follows. A heated, temperature-dependent resistor is suspended in the fluid stream. The temperature and thus resistance of the heated bead is proportional to the cooling effect of the fluid moving over its surface; the faster the flow the greater the cooling. A constant voltage is applied to the circuit and changes in resistance are monitored as voltage changes across a stable resistor in series with the thermistor. This voltage was compared to a control circuit and plotted directly as a voltage on the chart recorder.

The flow probe was a polyethylene T piece with two temperature-dependent resistors protruding into the lumen, their surfaces being siliconised (see figure III.2). An outer nylon sheath was cemented around the probe to provide mechanical support. A cyano-acrylate adhesive (Loctite) was used throughout the construction and further support was added with epoxyresin (Araldite). Ventral aortic pressure was measured through the cannula (Portex PP160) which opened into the centre of the probe. The upstream thermistor (Phillips 3K3 NTC resistor) was used to record blood temperature while the downstream thermistor (Phillips 1K5 NTC resistor) was heated and measured flow. Power output of the bead was 30.5 mW at 15°C. The change in blood temperature as measured by the upstream thermistor after activation of the flow meter was 0.2 ± 0.093°C (n = 14).

FIGURE III.2 Diagram of flow probe. A constant voltage was applied across the downstream thermistor. The current which passed through this temperature dependent resistor heated the bead. Fluid flow over the surface of the bead cooled the resistor producing flow dependent changes in resistance. Changes in resistance were recorded as voltage changes across a stable resistor in series with the thermistor. The upstream thermistor was used to record blood temperature. d.t, downstream thermistor; u.t, upstream thermistor.


ut
dt
1
5mm

The flow probe was calibrated against three fluids. The first was eel Ringer's solution with 10 g l^{-1}PVP (MW 360,000, Sigma Chem. Co.) added to create a viscosity approximately halfway between that of short-finned eel blood and plasma. The second fluid was a 13 g l^{-1} solution of PVP in water, approximating the bulk viscosity of eel blood. In the third case the flow probe was perfused with 45 ml of freshly collected eel blood. Fluid was delivered with a peristaltic pump at three different stroke volumes (Cole Parmer, Masterflex) and flow rates were measured gravimetrically at the outflow. The fluid reservoir, flow probe and heat exchange coil were immersed in a constant temperature water bath during calibration. Flow rates in ml min^{-1} were plotted against voltage at 0.5°C intervals between 15°C and 18°C. Nomograms were constructed from the isothermal flow/voltage profiles, where the displacement of the curve on the voltage axis was directly proportional to the ambient temperature. Thus for a given temperature, measured with the probe, the voltage reading corresponds to a particular flow rate in ml min^{-1}.

The curve was hyperbolic, asymptotic to infinite flow and zero voltage and was therefore less accurate at flow rates below 1.0 ml min^{-1} and above 24 ml min^{-1}. The accuracy of the flow probe was estimated over a range of pump delivery rates encompassing the flows recorded in the fish. The flow calculated from the nomogram was compared to gravimetric flow. The mean error was 4.86% and 95% of the calculated flows fell within 12.2% of the gravimetric value. No detectable difference was observed on substitution of 13 g l^{-1} PVP solution for FW eel Ringer's solution with 10 g l^{-1} PVP added. Eel blood pumped through the probe in one trial produced an apparent decrease in flow of 3.5%.

To guard against the possibility that fibrin deposition on the resistor bead might alter the sensitivity of the flow probe it was taken from two fish after trials and its performance was tested immediately after exposure to the fish's blood. There was no displacement of the calibration curve. Additionally, microscopic checks gave no evidence of fibrin deposition.

The presence of the flow probe in the ventral aorta will contribute to the total resistance of the vascular system. The pressure differential across the flow probe was determined under conditions of constant pulsatile flow and the total resistance calculated to be 0.059 kPa ml min^{-1}.

III.2.4 Pressure measurement

Bell and Howell type 4-327-0010 transducers were used in the measurement of blood pressures. The cannulae were all c. 1.5 m in length. The performances of the transducers and cannulae were examined by the free-vibration method (McDonald, 1974). The resonant frequency of the ventral aortic transducer and cannula (Portex PP160) was 5.9 Hz with a damping of 39% of critical. The resonant frequency of the dorsal aortic transducer and cannula (Portex PP50) was 3.8 Hz and damping was close to 100% of critical. The caudal venous transducer and cannula (Portex PP60) had a tapered tip and was slightly overdamped. Thus while we could accurately record mean pressures in all three blood vessels cannulated the pulse pressures recorded for dorsal aorta cannot be considered reliable estimates.

III.2.5 Experimental protocol

With two exceptions recorded below, the fish were allowed 18 hr to recover from the effects of surgery. During experiments, pressures and flows were monitored continuously on a chart recorder (Devices MX4). Records were taken before exercise, when swimming against an imposed current and at rest after exercise. If during swimming trials an eel persistently failed to maintain station, the water velocity was reduced to the resting value and the fish allowed to recover. The earliest record of swimming was obtained 10 hr after the end of surgery, and the latest 48 hr after surgery.

For all fish branchial resistance (Kb) has been calculated by dividing the pressure differential between the ventral and dorsal aortas (ΔPg in kPa) by the cardiac output (Q in ml min^{-1}). Student's t-test was used to establish the significance of paired differences. Values are given as mean ± 1 sample standard error of the mean.

III.3 Results

Records were obtained from ten eels. Six eels responded to increases in water velocity by swimming and data presented here is from 14 trials using these fish. Fish were tested at two different swimming velocities. This results section is divided into five parts. In part III.3.1 data from resting eels before swimming is presented. In part III.3.2 data derived from eels swimming at 15 cm s^{-1} is presented. This corresponds to a swimming speed of around 0.25 body lengths per second. Part III.3.3 describes data from fish swimming at 22-25 cm s^{-1}. Following the test period one fish from each group, in two separate trials, was swum to apparent exhaustion at increased water speeds. Part III.3.4 describes cardiovascular changes after swimming stopped. As there were marked changes in the calculated values of Kb with time, the results are plotted on a logarithmic scale to facilitate comparisons. Part III.3.5 describes the effects of administration of catecholamines to two fish.

III.3.1 Resting values

Mean values are given in table III.1 of pressures and flows at rest in the fish used in the 14 swimming trials. Four fish did not swim in response to increased water current. Their values for Kb in kPa ml^{-1}min were 0.040, 0.096 and 1.98 and 4.05. It is noted here that Kb for fish which swam ranged from 0.154 kPa ml^{-1}min to 0.692 kPa ml^{-1} min.

III.3.2 Swimming at 15 cm s^{-1}

When fish started to swim there was a brief bradycardia for about 60 s and with this an associated fall in Q which subsequently stabilized. In this group there were no significant changes in Kb, Q or ΔPg with time (see figure III.3 and appendix A.41). In five of the six eels there was a fall in Kb evident at five minutes. This was caused by increased cardiac output (increased stroke volume), and a fall in ΔPg.

In the two trials in which fish 24 was swum to apparent exhaustion at 22 cm s^{-1}, there was an increase in Kb. At the end of this period the fish was unable to move from the downstream grid.

Table III.1 Mean resting values of cardiovascular parameters ± 1 S.E.M. in short-finned eels (n = 14).

Parameter	Value	± 1 S.E.M.
Kb kPa $ml^{-1}min$	0.338	0.043
Q $ml^{-1}min$	6.87	0.821
Q ml $min^{-1}kg^{-1}$	11.43	1.35
Heart rate beats min^{-1}	51.07	1.33
Stroke volume ml	0.137	0.017
Stroke volume ml kg^{-1}	0.226	0.027
VAP kPa	5.12	0.23
DAP kPa	3.05	0.11
CVP kPa	0.844	0.082
ΔPg kPa	2.07	0.30
ΔPt kPa	2.20	0.11

III.3.3 Swimming at 22-25 cm s^{-1}

In this group there were significant increases in Kb after 5, and 30 minutes of swimming ($P < 0.05$). The rise in Kb was caused by an increase in ΔPg and a fall in Q (table III.2 and appendix A.42). The increase in ΔPg was due to a rise in VAP while DAP fell slightly. During these trials, with marked changes in ΔPg, there was little change in ΔPt. On autopsy it was found that fish 29' had clotted blood in the flow probe, in which case we might presume that a restricted Q exaggerated the rise in Kb.

In four trials the fish stopped swimming and did not move from the downstream grid and in three of these cases this occurred after the fish was subjected to an increased water current (35 cm s^{-1}). The points at which the fish stopped swimming marked the highest recorded values of Kb, as was the case when fish stopped swimming spontaneously at lower velocities (see figure III.4). In the nine trials where the fish

FIGURE III.3 Branchial resistance versus time for fish swum at 15 cm s^{-1}. Star indicates fish swum to apparent exhaustion. No prime indicates first trial, one prime second trial, two primes third trial. (See appendix A.42.)

——— 15 cm s^{-1}

----- 25 cm s^{-1}

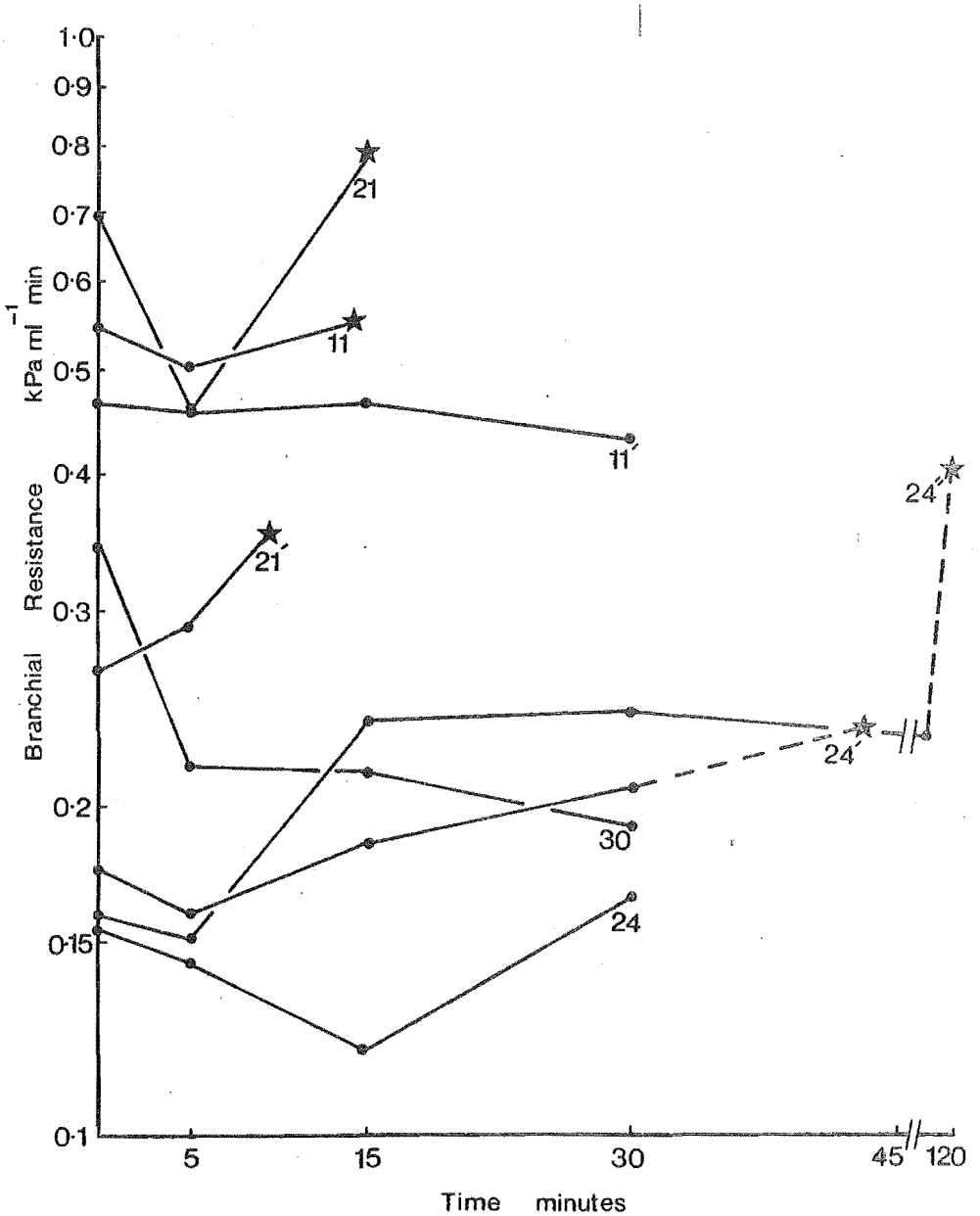

1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·15
0·1
Branchial Resistance kPa ml⁻¹ min
21
11
11′
21′
24′
24′
30
24
5
15
30
45
120
Time minutes

Table III.2 Mean values of cardiovascular parameter ± 1 S.E.M. during swimming at 22-25 cm s^{-1} (n = 6).

	Kb kPa $ml^{-1}min$	Q ml min^{-1}	ΔPg kPa	VAP kPa	DAP kPa
Resting values	0.322 ± 0.0405	6.79 ± 1.20	2.23 ± 0.300	5.26 ± 0.510	3.03 ± 0.223
15 min swimming	0.534 ± 0.0746	6.43 ± 1.16	3.33 ± 0.609	6.24 ± 0.486	2.91 ± 0.202
Significance n = 6	s; $P < 0.05$	NS	NS	NS	NS

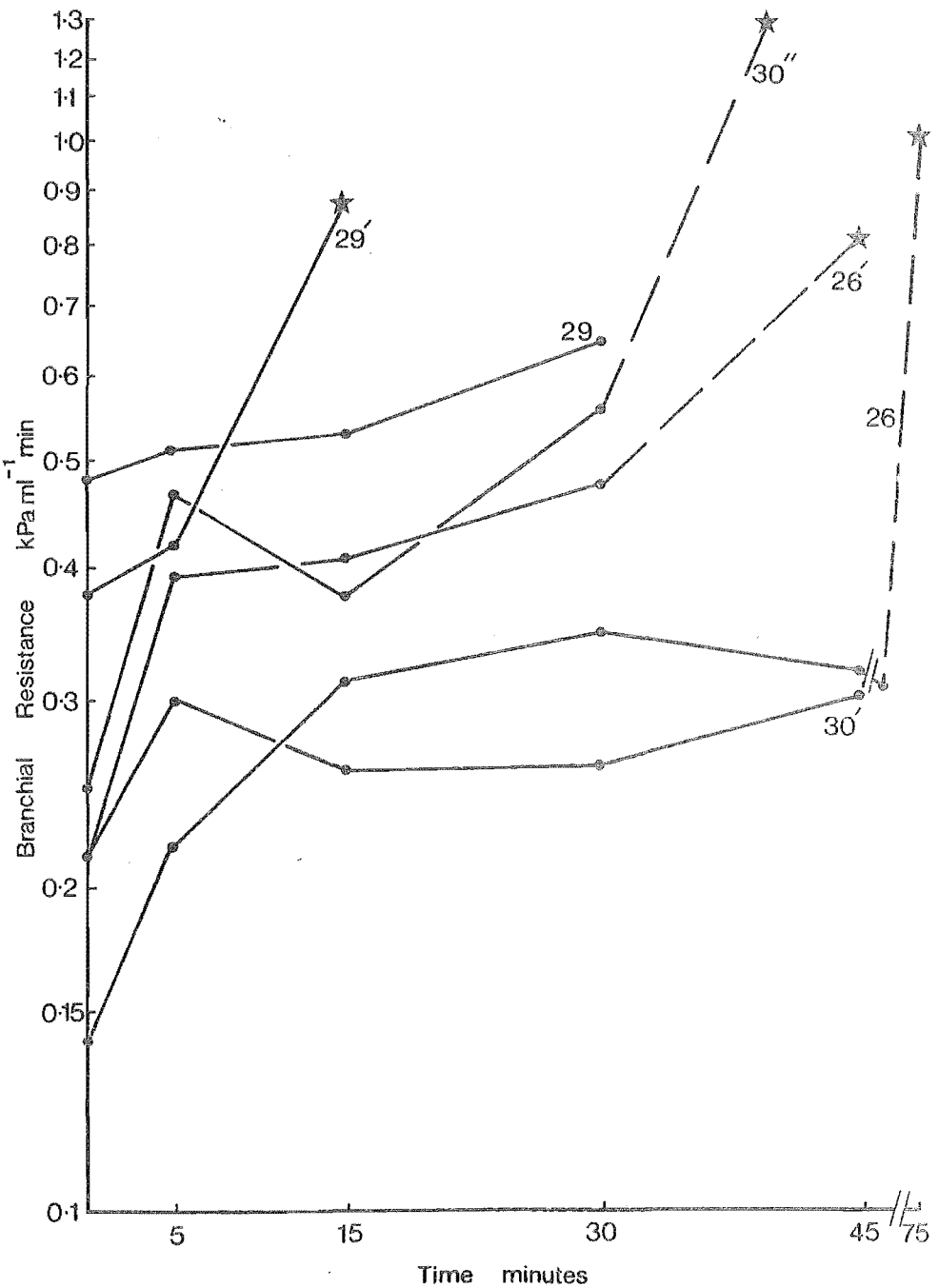
Table III.3 Mean values of cardiovascular parameters ± 1 S.E.M. after swimming trials (n = 12).

	Kb kPa $ml^{-1}min$	Q ml min^{-1}	ΔPg kPa	VAP kPa	DAP kPa
Values at end of swimming	0.555 ± 0.084	6.25 ± 0.831	2.96 ± 0.390	6.01 ± 0.335	3.06 ± 0.126
15 min rest	0.382 ± 0.0696	7.18 ± 1.06	2.25 ± 0.335	5.33 ± 0.255	3.08 ± 0.127
Significance n = 11	s; $P < 0.01$	s; $P < 0.05$	s; $P < 0.05$	s; $P < 0.05$	NS

FIGURE III.4 Branchial resistance versus time for fish swum at 22-25 cm s^{-1}. Star indicates fish swum to apparent exhaustion. No prime indicates first trial, one prime second trial, two primes third trial. (See appendix A.43, and table III.2).

—— 22-25 cm s^{-1}

----- 35 cm s^{-1}


1·3
1·2
1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·15
0·1
Branchial Resistance kPa ml⁻¹ min
30″
29′
29
26′
26
30′
5
15
30
45
75
Time minutes

were washed onto the downstream grid after a period of swimming this occurred after a significant rise in ΔPg (ΔPg at rest = 1.97 ± 0.189 kPa rising to 3.15 ± 0.340 kPa; $P < 0.05$) and a significant fall in Q (Q at rest = 6.85 ± 0.855 $ml^{-1}min$ falling to 5.31 ± 0.851 $ml^{-1}min$; $P < 0.05$).

III.3.4 Recovery from swimming

In this section results were pooled from all trials apart from the second trials of fish 21, which displaced a cannula, and fish 29 which had a blood clot in the flow probe. At the end of swimming some fish were apparently exhausted, some stopped swimming spontaneously while others were still swimming vigorously.

After swimming ceased there was a significant fall in Kb (see figure III.5). Kb fell from 0.555 ± 0.084 kPa $ml^{-1}min$ at the end of swimming to 0.394 ± 0.0742 kPa $ml^{-1}min$ after five minutes rest, ($P < 0.01$, $n = 12$). Thereafter there was little change in Kb. The fall in Kb was due to a significant fall in ΔPg once again due to a change in VAP rather than DAP (table III.3 and appendix A.43). There was also a significant increase in Q. At this time there was an averaged 5% increase in heart rate but once again changes in Q were largely achieved by changes in ventricular stroke volume.

III.3.5 Administration of catecholamines

The stress of surgery and handling could have elevated blood catecholamine levels (see Mazeaud, et al., 1977), or alternatively the secretory capacity of the chromaffin tissue might be exhausted. To test this possibility eels 24 and 26 were injected with catecholamines at the end of their final period of recovery from swimming.

Fish 24 was given an injection of adrenaline (1.8 µg) and atropine (1.6 mg) in eel saline solution (0.2 ml), via the caudal vein. The dose of adrenaline was calculated to produce a blood concentration of about $5 \times 10^{-7}M$, which is the dose that produces a half-maximal constrictory response of the isolated perfused eel tail (see table II.3). The results of this treatment are shown in figure III.6. The absence of irregularities in the trace are probably due to the blocking action of atropine at cholinergic neuromuscular junctions, including those of the opercular muscles.

VAP rose by 48.6% and DAP by 49.1%, reaching peak values within 90 s of injection. As the absolute rise in VAP was greater than that found in the DAP, ΔPg was increased by 0.933 kPa. Cardiac output fell

FIGURE III.5 Branchial resistance versus time for fish recovering from swimming at both speeds. All fish showed falls in branchial resistance within the first five minutes. This fall is most pronounced in fish swum to apparent exhaustion (dotted lines) (see appendix A.44 and table III.3).

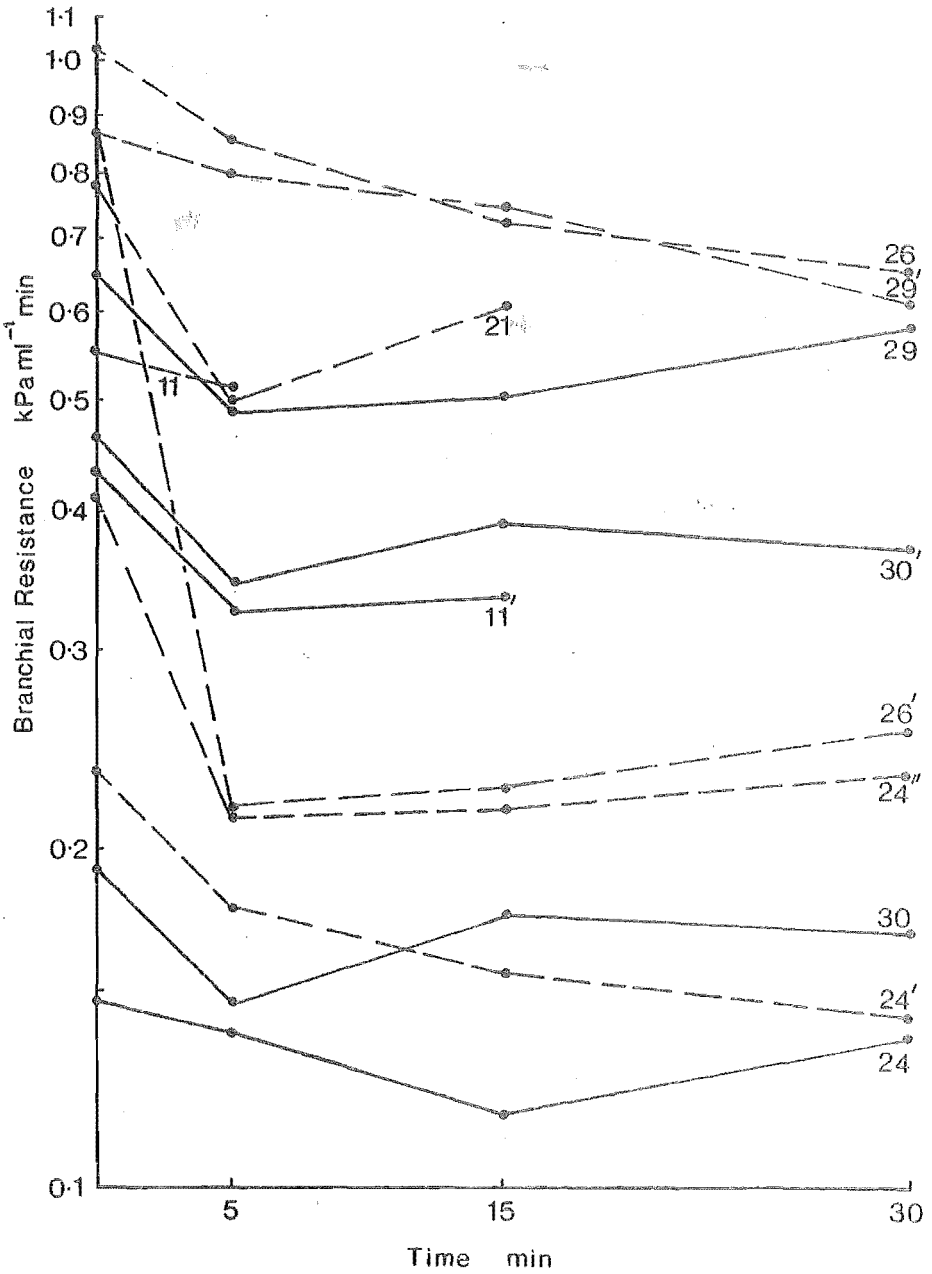
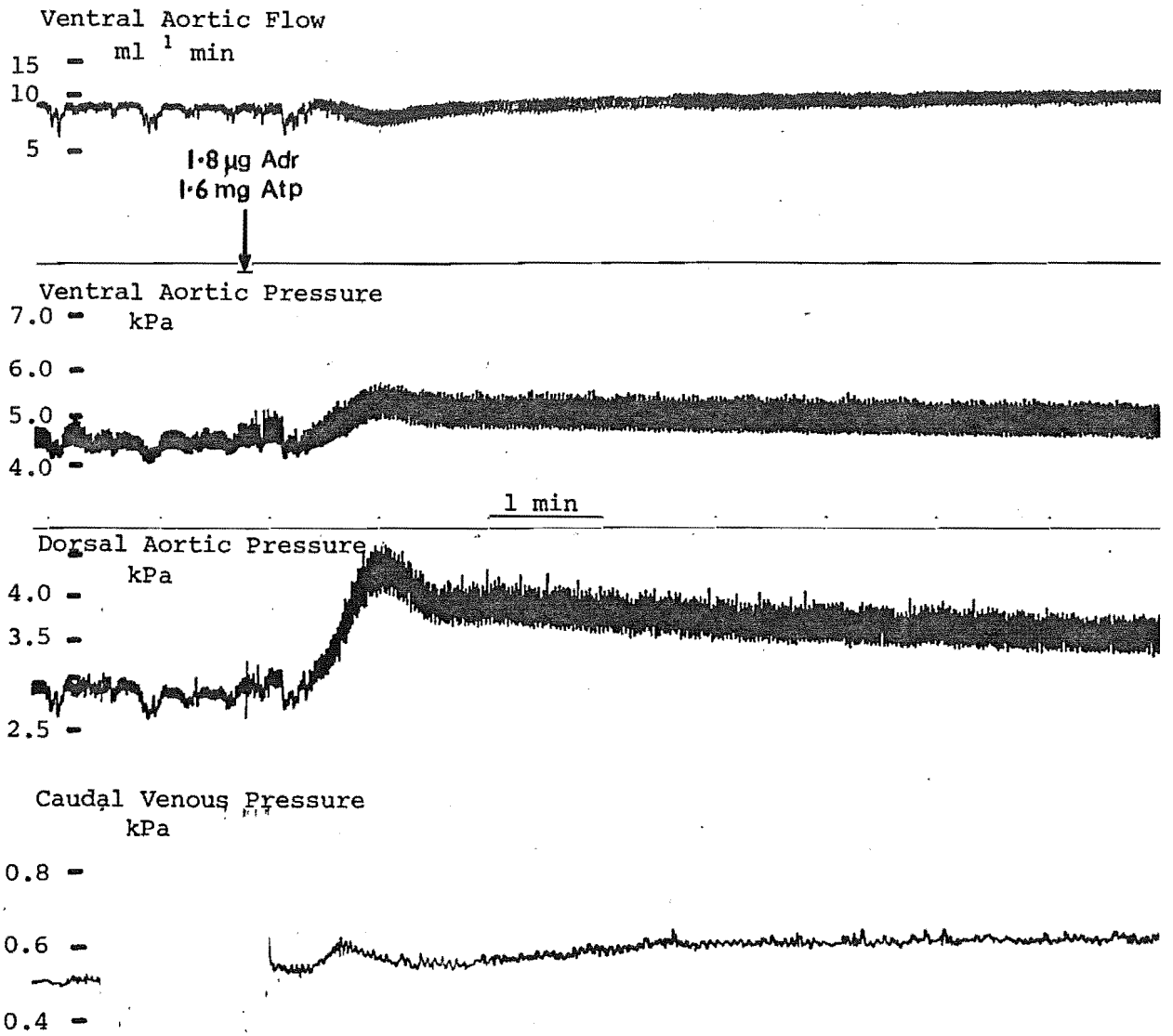

1·1
1·0
0·9
0·8
0·7
0·6
0·5
0·4
0·3
0·2
0·1
Branchial Resistance kPa ml⁻¹ min
5
15
30
Time min
26
29′
21
29
11
30′
11″
26′
24″
30
24′
24

FIGURE III.6 Record of response to administration of 1.8 µg adrenaline plus 1.8 µg atropine into fish number 24 two hours after its last swimming trial via the caudal vein. Cardiac output fell slightly while ventral and dorsal aortic pressures increased by about 50%. Both branchial resistance and tail pressure differential increased (see section III.3.5).


Ventral Aortic Flow
ml min⁻¹
15
10
5
1·8 µg Adr
1·6 mg Atp
Ventral Aortic Pressure
kPa
7.0
6.0
5.0
4.0
1 min
Dorsal Aortic Pressure
kPa
4.0
3.5
2.5
Caudal Venous Pressure
kPa
0.8
0.6
0.4

from 9.9 to 8.4 ml min^{-1}. Thus Kb rose from 0.202 to 0.323 kPa ml min^{-1}. ΔPt increased from 2.86 to 4.21 kPa, due to the increase in DAP. Heart rate rose from 46 to 55 beats per minute.

Eel 26 was injected with 3.6 µg of isoprenaline in 0.2 ml of eel saline solution, a dose calculated to give a half maximal dilatory response in the eel caudal circulation (see table II.3). Cardiac output rose to a maximal 25% increase at 2 min. ΔPg did not change, though VAP and DAP both fell slightly. Thus Kb fell from 0.236 to 0.189 kPa ml^{-1} min.

At 5 min cardiac output had dropped to 13% below the initial value. At this time VAP and DAP reached their minimal values, with a relatively greater fall in VAP. Thus ΔPg was reduced and Kb was calculated to be 0.224 kPa ml^{-1}min, which is below the initial level despite the drop in cardiac output which would tend to cause a rise in Kb. Caudal venous pressure decreased by 19.3% in response to isoprenaline, with a 25.7% decrease in ΔPt.

III.4 Discussion

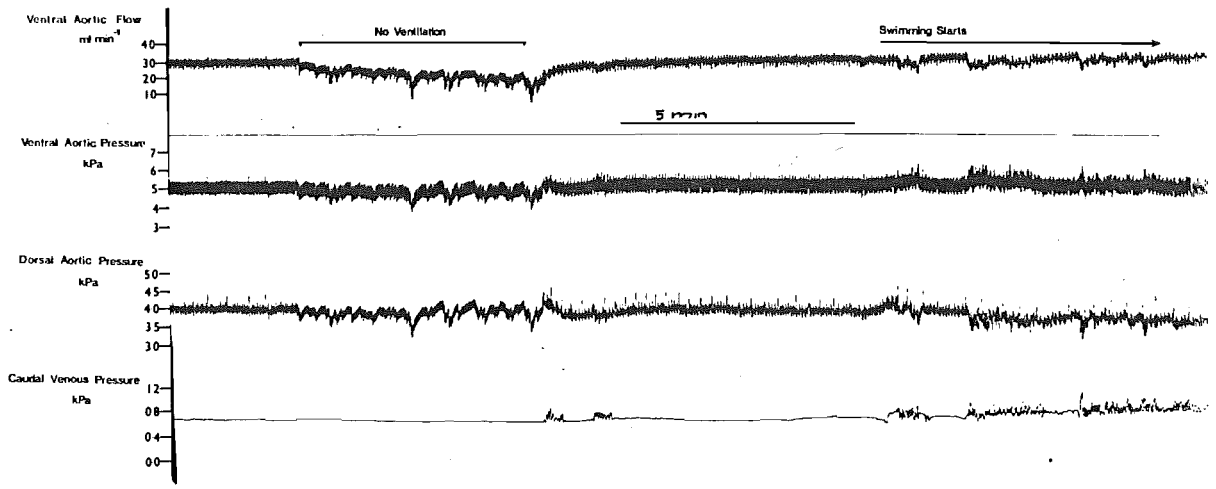
Fish in these experiments underwent major surgery and anaesthesia. The physiological effects of handling and anaesthesia may still be apparent up to 48 hrs after surgery. In all but two cases results were obtained at least 18 hr after surgery, a post-operative period similar to that adopted by Kiceniuk and Jones (1977). From early trials it was apparent that fish could not swim within 10 hr after the end of anaesthesia. Two of our experimental fishes exhibited periodic ventilation (figure III.7; see van Dam, 1938; Shelton and Randall, 1962). This phenomenon is characteristic of intact resting short-finned eels, in which the periods without opercular movements increase in frequency and length under hyperoxic conditions. Thus fish exhibited a normal pattern of ventilation and it is unlikely that they were subjected to chronic hypoxaemia.

The 1.5 m lengths of cannula used in our experiments allowed the fish freedom of movement throughout the length of our experimental chamber, but will have increased the drag experienced when swimming. The highest sustainable swimming speed of 35 cm s^{-1} compares with a maximum reported swimming speed of 45 cm s^{-1} in the american eel (*Anguilla rostrata*), (Lagler, Bardach, Miller and Passino, 1977), and a speed of 51 cm s^{-1} for migrating silver european eels (Tesch, 1978).

We have calculated Kb from the ΔPg measured between the VA and DA and have assumed that blood passing through the gill emerges from the DA. As there is an alternative venous pathway for blood returning to the heart it is possible that changes in resistance of this second parallel circuit will influence the first (see Vogel, Vogel and Kramers, 1973). We have assumed that changes in resistance of the cephalic venous system will not significantly alter Kb.

The flow probe replaced part of the ventral aorta. The elasticity of the prebranchial circulation in fish plays an important part in smoothing blood flow through the gills (Satchell, 1971). However, it is the distensibility of the bulbus arteriosus which makes the major contribution to the compliance properties of this part of the blood system (see Licht and Harris, 1973; Priede, 1976). The presence of the flow probe may also contribute to our measurements of resistance to blood flow. The resistance of the flow probe was measured and found to be small in relation to Kb (mean Kb = 0.372 kPa ml^{-1}min. K flow probe = 0.059 kPa ml^{-1}min). Half of the probe resistance might be subtracted from the value of Kb reported, assuming the length of ventral aorta replaced has negligible resistance.

FIGURE III.7 Record of apnoea from fish 24 before swimming trial. There was a gradual fall in cardiac output associated with apnoea. Toward the end of the record presented here, the fish was subjected to increased water current which initiated swimming. The effects of muscular contractions on pressures is particularly evident in the record of caudal venous pressure.


Ventral Aortic Flow
ml min⁻¹
40
30
20
10
No Ventilation
Swimming Starts
5 min
Ventral Aortic Pressure
kPa
7
6
5
4
3
Dorsal Aortic Pressure
kPa
50
45
40
35
30
Caudal Venous Pressure
kPa
12
08
04
00

It is possible that movements associated with swimming may have changed the pattern of flow in the ventral aorta and within the implanted flow probe. One advantage of the probe that we used is that the rigid walls of the probe should maintain constant cross-sectional area at the position of the heat sensitive resistors. In this respect it is important to note that when the transient bradycardia found at the beginning of exercise is disregarded, the change in cardiac output recorded in swimming fish was gradual and not abrupt as might be expected were mechanical artefacts to intervene. Additionally any restriction to flow caused by ventilatory movements would have been suddenly released at the start of apnoeic periods. As can be seen in figure III.7, there was a gradual fall in Q during the apnoeic period, associated with bradycardia.

Values of cardiovascular parameters from resting fish reported here (table III.1) are comparable with results from previous workers. From data on trans-branchial pressure differentials (Helgason and Nilsson, 1973) and cardiac output (Johansen, 1962), Kb of the cod was calculated to be around 0.207 kPa $ml^{-1}min$. Branchial resistance of the lingcod was reported to be 0.458 kPa $ml^{-1}min$ (Stevens et al., 1972). Rainbow trout Kb calculated from the data of Stevens and Randall (1967a, b) was 0.151-0.226 kPa $ml^{-1}min$. All of these estimates are in agreement with the range of Kb observed in the short-finned eel. Kiceniuk and Jones (1977) however reported rainbow trout Kb at rest to be 0.059-0.062 kPa $ml^{-1}min$, which is considerably lower than estimates in this and other species.

The ventral and dorsal aortic pressures of fresh water eels given by Chester-Jones, Chan and Rankin (1969) and Chan and Chow (1976) are similar to those reported here. Chan and Chow (1976) gave a post-cardinal venous pressure 0.12 kPa compared to the mean CVP of 0.844 kPa reported here, the difference probably reflecting the pressure drop across renal and hepatic portal vascular beds. Thus the estimates of Kb, Q and pressures measured in the short-finned eel are comparable to reported values from other teleost species and are thought to be realistic.

The most striking response to swimming in these fish was the rise in ventral aortic pressure which, when combined with decreased cardiac output and the relatively stable dorsal aortic pressure, caused the observed increase in Kb.

Laurent and Dunel (1976) have described the morphology of the blood vessels of the gill filament of the European eel. The central venous

sinus differs from that of trout and perch in that it has extensions surrounding the primary afferent artery. Engorgement and increased pressure in the central venous sinus could in turn increase pressure in the afferent filamental arteries. In the secondary lamellae, the blood water respiratory distance is about 6 μm (Steen and Berg, 1966) and a rise in VAP during exercise may threaten their competence. At very high Kb swimming ceased. This response may have a protective function for the gills. Blood pressures in the ventral aortas of teleosts are high in relation to pulmonary artery pressures of tetrapods (Johansen, 1972), and yet the blood to water distance at the gill lamellae can approach the values for mammalian alveoli (Steen and Berg, 1966; Hughes and Morgan, 1973).

Caudal venous pressure showed large fluctuations associated with swimming movements (see figure III.7). Low pressures make the venous system more susceptible to changes in transmural pressures from swimming muscles and could augment blood return to the heart (Satchell, 1965).

Kiceniuk and Jones (1977) have published cardiovascular changes associated with swimming in rainbow trout which are in marked contrast to the results presented here. These authors reported a threefold increase in Q and a fall in Kb. That they recorded very low Kb values compared to other data from this and other species has already been noted. However they had a more rigorous pre-surgery training schedule. In view of the differences in body form and behaviour between the trout and the eel, and the clearly different patterns of cardiovascular responses to exercise, an in depth comparison is unwarranted. A better comparison might be made between cod or lingcod and eels. Stevens et al. (1972) reported pressures and flows in the lingcod that agree well with our results. The trace of the responses to spontaneous swimming in the lingcod (figure 7 of Stevens et al., 1972) is very similar to these results (see figure III.7), including the bradycardia at the start of swimming. Likewise the report by Johansen et al. (1960) shows that the responses of the heart of some elasmobranchs to swimming are very similar to eel heart responses, particularly the rise in ventral aortic blood flow after exercise ceased. The overall pattern of cardiovascular responses to swimming in trout differs from that described here in eels and previously reported accounts in lingcod and elasmobranchs. These differences and the absence of any well defined beta dilatory adrenergic response in the trout trunk vasculature indicate that the teleost cardiovascular system is not typified by that of the rainbow trout.

Experiments on two eels after the end of swimming trials showed that systemic blood vessels still possessed the ability to respond to exogenous catecholamines (section III.3.5). When combined with the responses of the eel to swimming, it is evident that plasma catecholamine concentrations did not rise under the conditions of these experiments. Plasma catecholamines may have a physiological role in the stress response but not in exercise.

The reasons for the observed changes in the cardiovascular system and behaviour during swimming seems to arise from the need to protect the gills from very high pressures. Cessation of swimming in response to branchial oedema has been suggested by Satchell (1978) from studies on the dogfish (*Squalus acanthias*). Since higher vascular pressures would result in extravasation of vascular fluid (Landis and Pappenheimer, 1963) protection of the gills seems a logical explanation for these results. The reduced cardiac output observed on swimming is surely incompatible with the greater metabolic requirements of exercise.

III.5 Summary

1. Cardiac output, ventral aortic pressures, dorsal aortic pressures and caudal venous pressures were recorded from unrestrained short-finned eels at rest, swimming at 15 $cm.s^{-1}$ and 22-25 $cm.s^{-1}$ and after exercise.
2. Pressures and flows and calculated values for branchial resistance at rest are in good agreement with previously reported values (section III.3.1).
3. Swimming at 15 $cm.s^{-1}$ resulted in insignificant changes in branchial resistance. After five minutes of swimming at this speed there was a fall in branchial resistance caused mainly by a rise in cardiac output. Thereafter little change in branchial resistance was recorded (section III.3.2).
4. Swimming at 22-25 $cm.s^{-1}$ significantly increased branchial resistance principally as a result of elevated ventral aortic pressures. Changes in heart rate, dorsal aortic pressures and caudal venous pressures were small. Cardiac output fell slightly (section III.3.3).
5. Changes in cardiac output were achieved largely through changes in stroke volume rather than heart rate.
6. After swimming ceased, branchial resistance fell as a result of increased cardiac output and decreased ventral aortic pressure (section III.3.4).
7. Plasma catecholamine levels appear to be unimportant in the cardiovascular responses to swimming in the short-finned eel.
8. Spontaneous cessation of swimming was associated with very high branchial resistances. This response may have a protective function for the gill tissues.

PART IV SUGGESTIONS FOR FURTHER WORK

During the course of this study, particularly in the latter stages, it has become clear that further research into several aspects could significantly contribute to our understanding of fish cardiovascular physiology. The more interesting, and possibly more fruitful avenues as I see them are noted below.

1. The caudal lymph heart of the eel is controlled by a unique example of a vertebrate neural pacemaker. It has relatively few neural elements (for a vertebrate), is easily isolated and with the appropriate micro-electrophysiological techniques would provide a useful comparison for the vertebrate cardiac pacemaker and possibly invertebrate neural pacemaker systems. Pharmacological analysis of this organ would complement the above study.
2. The basic adrenergic pharmacology of the isolated saline perfused eel tail has been described in this report. On this foundation an investigation of the cholinergic and perhaps purinergic vascular pharmacology could be undertaken to allow comparison with the work on the trout trunk.
3. As demonstration of the beta dilatory response in the trout trunk was difficult to demonstrate, one wonders how the responses might be enhanced. Clearly one method would be the use of human serum in the perfusate. Another might be the isolation of the vessels that contain more beta receptors. Muscle vessels seem to be good candidates and perhaps better still, the vessels from red muscle since it is these muscles that operate aerobically during swimming. Furthermore it might be better to choose fish which are mature and ready to migrate for it is in this condition that they should be physically ready to swim. The use of immature 'feeding' fish in the past may have resulted in the selection of fish where systemic vascular dilation was relatively undeveloped.
4. This preparation is well suited to study the effects of urophysial secretions on the caudal circulation. Inclusion of the kidney into the preparation may make it even more suitable since no isolated perfused fish kidney preparation is available to fish physiologists as far as I am aware.
5. Cardiovascular responses to swimming in fish are far from well described, let alone understood. Results presented here show marked differences to those from trout and yet agree well with results from

various other fishes. More data on basic changes in pressures and flows are needed to establish what changes do occur and the cause of the observed responses.

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Appendix A.1 ΔK responses (kPa $ml^{-1}min$) to AD. Perfusion line. No serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
A101	0	0	0.0951	0.0938		0.1251		0.9651		1.629	1.662	1.662
A102	0	0	0.1036	0.3564		0.7786		2.074		2.718	2.240	2.240
A103	0	0.0876	0.1607	0.2558		0.4825		2.018		2.630	2.637	2.637
A104	0	0.0535	0.0713	0.2676		0.3121		2.311		3.772	3.769	3.769
A105	0.0623	0.1824	0.2284	0.2826	0.3422	0.4362	1.0621	1.483	2.347	2.381	2.415	2.415
A106					0.3181		0.8235		1.849			
mean	0.0124	0.0647	0.1318	0.2517	0.3303	0.4261	0.9048	1.774	2.100	2.629	2.546	2.546
1 S.E.	±0.0124	±0.0338	±0.0281	±0.0429	±0.0171	±0.0959	±1.1599	±0.2173	±0.3526	±0.3074	±0.3081	±0.3081

Appendix A.2 ΔK% responses to AD. Perfusion line. No serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
Al01	0	0	9.86	9.49		12.96		101.22		168.80	172.61	172.61
Al02	0	0	11.11	38.19		83.33		222.22		293.05	240.27	240.27
Al03	0	8.33	15.27	24.28		45.83		193.05		249.99	249.99	249.99
Al04	0	5.0	6.66	25.00		29.16		215.83		315.16	315.16	315.16
Al05	8.10	22.97	29.73	36.81	44.59	56.75	128.37	193.34	305.46	309.45	316.21	316.21
Al06					83.72		216.28		485.93			
mean	1.62	7.25	14.52	26.75	64.15	45.61	172.32	185.13	395.69	267.09	258.82	258.82
1 S.E.	±1.62	±4.23	±4.04	±5.19	±19.56	±12.00	±43.95	±21.78	±90.23	±27.17	±26.76	±26.76

Appendix A.3 ΔK responses (kPa $ml^{-1}min$) to AD. Perfusion line. Plus 5% serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
A201	0.1593	0.5607	0.5088	0.6678		1.0813		1.5901		2.8463	3.4823	3.4823
A202	0.1684	0.3649	0.3312	0.3874		0.8669		1.2848		2.4537	2.4537	3.6151
A203	0	-0.1138	0.2134	0.5334		1.3233		2.4622		2.7889	2.8450	2.8450
A204	0.0307	0.0799	0.8605	0.2335	0.3811	0.5532	1.6105	2.2005	3.0795	3.1226	3.5681	3.3070
A205	-0.0992	-0.0555	0.0297	0.1092	0.3078	0.5858	1.5488	1.9163	2.3135	2.4724	2.7008	2.6610
A206						1.1112		3.2401		4.8199	3.5747	3.5747
A207					0.5148		1.5449	1.6500	24.2813			
ADB201							1.8454					
ADB202							3.3383					
ADB203							1.0851					
ADB204							1.3628					
ADB205							2.2138					
mean	0.05176	0.1552	0.2338	0.3863	0.4012	0.9746	1.8187	2.0583	2.6072	3.0839	3.1041	3.1475
1 S.E.	±0.0366	±0.0888	±0.0863	±0.1003	±0.0494	±0.1260	±0.3487	±0.2434	±0.2065	±0.3620	±0.2027	±0.1489

Appendix A.4 ΔK% responses to AD. Perfusion line. Plus 5% serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
A201	6.90	22.22	22.22	29.16		47.22		69.44		124.31	152.08	152.08
A202	6.77	14.67	13.31	15.15		34.08		54.26		98.64	98.64	121.21
A203	0	-5.19	9.74	24.35		60.38		112.33		127.27	129.87	129.87
A204	3.70	10.16	10.93	29.68	48.43	70.31	204.68	279.68	391.40	396.87	453.1	420.30
A205	-10.64	-5.31	3.56	13.02	37.23	62.76	185.37	205.31	247.86	264.88	289.35	285.10
A206						45.23		131.88		196.18	145.50	145.50
A207					52.57		161.34	183.00	253.61			
ADB201							107.27					
ADB202							145.38					
ADB203							94.16					
ADB204							63.21					
ADB205							166.66					
mean	1.34	7.31	11.95	22.27	46.07	53.33	141.01	140.84	297.62	201.35	211.42	209.01
1 S.E.	±3.24	±5.48	±3.03	±1.39	±5.95	±5.48	±17.16	±29.67	±61.01	±46.29	±55.29	±48.88

Appendix A.5 ΔK responses (kPa $ml^{-1}min$) to AD. Bolus. No serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
A151		0	0.3810	0.6792	0.8447	1.2590	1.7725	2.3523	3.5451	4.0753
A152	0	0.0297	0.1040	0.2318	0.4624	0.6129	0.7432	1.1150	2.3861	3.0476
A153	0	0.0741	0.0741	0.1112	0.4171	0.3526	0.8527	1.2421	2.3541	3.9487
A154	0	0	0.3970	0.7036	1.1909	1.6780	2.3457	2.5261	4.2224	5.0885
A155	0	0	0.3491	0.9652	1.6225	1.7457	1.9716	2.9234	3.3476	5.8093
mean	0	0.0207	0.2510	0.5382	0.9435	1.1298	1.5394	1.9317	3.1711	4.3938
1 S.E.		±0.0145	±0.0665	±0.1591	±0.2274	±0.2801	±0.3161	±0.3096	±0.3578	±0.4797

Appendix A.6 ΔK% response to AD. Bolus. No serum

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
A151		0	19.76	35.23	43.83	65.32	91.95	122.03	183.90	211.41
A152	0	3.81	13.36	26.41	52.83	69.81	95.41	143.12	306.28	391.20
A153	0	5.88	4.99	7.50	28.13	23.75	57.50	83.75	158.75	266.25
A154	0	0	16.17	28.67	48.53	68.38	95.59	102.94	172.07	207.36
A155	0	0	15.67	43.34	72.86	78.39	88.53	108.82	150.33	261.00
mean	0	1.94	13.98	28.23	49.23	60.73	85.79	112.12	194.27	267.44
1 S.E.		±1.23	±2.47	±5.96	±7.23	±9.42	±7.19	±9.93	±28.58	±33.25

Appendix A.7 ΔK response (kPa $ml^{-1}min$) to AD. Bolus. Plus 5% serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
A251	0.04984	0.1352	0.1423	0.2847	0.4556	0.5837	0.9682	1.1177	1.8937	2.5712
A252	0	0.1853	0.3280	0.7060	0.9983	11.1694	1.7257	1.9176	2.5101	4.4070
A253	0.04032	0.1288	0.2185	0.3640	0.5656	0.6440	0.7785	1.0249	1.7642	2.2684
A254	0.02750	0.0990	0.1650	0.3411	0.4841	0.6491	0.8142	1.1663	1.7384	1.9915
A255	0.12216	0.2809	0.4642	0.7086	0.8552	1.1117		1.2217	1.5515	2.0157
mean	0.0479	0.1658	0.2636	0.4822	0.6678	0.8316	1.0715	1.2896	1.8916	2.6507
1 S.E.	±0.0212	±0.0319	±0.0595	±0.0942	±0.0197	±0.1270	±0.2216	±0.6602	±0.1641	±0.4516

Appendix A.8 ΔK% responses to AD. Bolus. Plus 5% serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
A251	2.81	7.63	7.63	15.27	24.42	31.29	51.90	59.92	101.52	137.83
A252	0	9.39	16.63	36.15	50.61	59.29	87.48	97.61	127.25	223.42
A253	2.06	6.58	11.17	18.62	28.93	32.94	39.82	52.43	90.25	116.03
A254	1.35	4.86	8.10	16.75	23.78	31.89	40.00	57.20	85.40	97.83
A255	9.85	22.66	37.43	57.14	68.96	89.65		98.52	125.12	162.56
mean	3.21	10.22	16.19	23.98	39.35	49.01	54.80	73.14	105.99	147.53
1 S.E.	±1.72	±3.19	±5.54	±8.12	±8.88	±11.45	±11.25	±10.25	±8.68	±21.84

Appendix A.9 ΔK responses (kPa $ml^{-1}min$) to NAD. Perfusion line. No serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
N109	0	0.3645	0.8815	1.1114	1.4947			2.1271		3.3144		
N110	0	0.3253	0.3635	0.5357	0.5931					1.0906		
N112	0.1262	0.1122	0.2526	0.3437	0.5822	0.6874	1.4245	2.0133	2.8761	3.6197	3.0865	2.4973
N113	0	0.0509	0.0892	0.4014	0.3632	0.5161		0.8220	0.9877	2.7974	3.4092	2.8739
N114	0.1546	0.2550	0.4317	0.4389	0.5393	0.5058	0.5728	0.8655	1.2835	1.3838	1.5929	1.3588
N115	0	0	0.1009	0.1682	0.2018	0.4878	1.1858	1.9428	3.5492	3.1960	4.1210	2.5231
mean	0.0468	0.1847	0.3488	0.4561	0.6290	0.5493	1.0611	1.5542	2.1741	2.5670	3.0524	2.4111
1 S.E.	±0.0298	±0.0617	±0.1191	±0.1451	±0.1838	±0.0464	±0.2538	±0.2917	±0.6176	±0.4359	±0.5326	±0.3297

Appendix A.10 ΔK% responses to NAD. Perfusion line. No serum.

Conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
N109	0	25.86	62.53	78.84	106.04			150.90		235.19		
N110	0	21.07	23.35	34.71	38.43					70.60		
N112	12.67	10.95	24.65	33.55	56.84	67.11	139.02	196.56	280.79	353.38	301.34	243.81
N113	0	3.60	6.30	9.90	20.40	29.03		46.23	55.55	157.34	191.75	161.64
N114	11.45	18.88	30.03	32.50	39.93	37.74	42.41	64.08	95.05	102.47	117.96	100.61
N115	0	0	8.57	14.28	17.14	41.43	100.71	164.99	301.42	271.42	349.99	214.28
mean	4.02	13.39	25.90	33.96	46.46	43.83	94.04	124.55	183.20	198.40	240.26	180.08
1 S.E.	±2.55	±4.18	±8.27	±9.97	±13.29	±8.18	±28.09	±29.41	±62.96	±43.94	±52.52	±31.47

Appendix A.11 ΔK responses (kPa $ml^{-1}min$) to NAD. Bolus. No serum.

Dose	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER									
N151			0	0.2894		0.6188			
N152		0	0	0.0481	0.1734	0.4239	0.5782	1.8505	3.0648
N153		0	0	0.0991	0.1298	0.4660	1.2496	4.8197	5.7507
N154		0	0.1752	0.3358	0.4786	0.5984	0.8966	2.0869	4.2582
N155	0	0	0	0.1746	0.3857	0.7208	1.2641	2.6806	5.0924
N156	0	0	0	0.0555	0.1220	0.1824	0.2934	0.8356	0.7972
N157	0	0.1107	0.2214	0.4164	0.3983	0.6699	1.0864	2.2092	
N158	0	0	0.0758	0.2226	0.2301	0.2734	1.0068	1.3519	2.5023
mean	0	0.0158	0.0590	0.2052	0.2781	0.4942	0.9107	2.2621	3.5776
1 S.E.		±0.0158	±0.0321	±0.0479	±0.0530	±0.0682	±0.1356	±0.4828	±0.7448

Appendix A.12 ΔK% responses to NAD. Bolus. No serum.

Dose	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER									
N151			0	8.11		17.31			
N152		0	0	2.20	7.95	19.44	26.51	84.85	140.54
N153		0	0	6.66	10.69	31.33	84.00	324.00	386.58
N154		0	11.51	22.06	31.44	39.31	58.90	137.11	279.82
N155	0	0	0	15.55	34.34	64.18	112.57	238.30	456.36
N156	0	0	0	3.82	8.42	12.57	20.23	57.41	56.71
N157	0	4.74	9.49	16.67	15.94	26.82	43.49	88.43	
N158	0	0	3.24	9.91	10.24	12.66	46.60	62.66	115.99
mean	0	0.68	3.03	10.62	17.00	27.95	56.04	141.91	239.33
1 S.E.		±0.68	±1.68	±2.43	±4.23	±6.14	12.33	±38.46	±65.51

Appendix A.13 ΔK responses (kPa $ml^{-1}min$) to ISO. Perfusion line. No serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
I101												
I102		-0.0048				-0.0966		-0.1111				
I103												
I104	0	-0.0300		-0.0451		-0.1205		-0.1654		-0.1804		
I105				-0.0230	-0.0806		-0.1959			-0.3225	-0.0461	+0.5243
I106	0	-0.0198	-0.0397	-0.0694	-0.1190		-0.2034	-0.2430	-0.1934	-0.1339	+0.3719	+0.9275
I107			-0.0263	-0.0526	-0.0895	-0.1579	-0.1790	-0.1579	-0.1053	0	+0.3264	+0.5896
I108	0	-0.0335	-0.0791	-0.0951	-0.1625	-0.1734	-0.1678	-0.1678	-1.6498	0	+0.8168	+1.4434
I109	0	-0.0259	-0.0442	-0.1106	-0.1106		-0.1327	-0.1548	-0.2212	-0.2212	+0.5694	+5.8015
mean	0	-0.0228	-0.0478	-0.0659	-0.1125	-0.1371	-0.1757	-0.1666	-0.1712	-0.1430	+0.4070	+0.8130
1 S.E.		±0.0050	±0.0112	±0.0133	±0.0143	±0.0175	±0.0123	±0.0174	±0.0247	±0.0519	±0.1286	±0.1730

Appendix A.14 ΔK% responses to ISO. Perfusion line. No serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
I102	0	-0.42				-8.36		-9.62				
I103												
I104	0	-4.32		-6.45		-16.16		-22.22		-24.24		
I105				-2.77	-7.86		-19.10			-31.46	-4.49	+50.56
I106	0	-2.51	-5.03	-8.80	-12.90		-21.02	-25.12	-19.99	-13.84	+38.46	+95.89
I107			-2.92	-5.84	-9.94	-17.54	-19.88	-17.54	-11.69	0	+36.25	+65.49
I108	0	-5.04	-11.76	-14.28	-23.52	-26.05	-25.21	-25.21	-15.96	0	+122.68	+216.80
I109	0	-2.78	-4.73	-11.34	-11.83		-14.20	-16.56	-23.67	-23.67	+60.94	+63.32
mean	0	-3.01	-6.11	-8.33	-13.21	-17.03	-19.88	-19.39	-17.83	-15.53	50.76	98.41
1 S.E.		±0.80	±1.94	±1.72	±2.72	±3.62	±1.77	±2.46	±2.58	±5.42	±19.86	±30.58

Appendix A.15 ΔK responses (kPa $ml^{-1}min$) to ISO. Perfusion line. Plus 5% serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
I201	-0.0561	-0.0748		-0.1309		-0.2712		-0.4208				
I202	-0.2612	-0.2612		-0.3294		-0.5565		-0.8292				
I203	-0.0408	-0.0830		-0.1295		-0.3223		-0.5755		-0.7091		
I205	-0.0476	-0.0692	-0.0692	-0.0953	-0.1428		-0.1559	-0.2122	-0.2252	-0.2252	-0.0606	+0.8489
I206	-0.0599	-0.0798	-0.0998	-0.1597	-0.6190	-0.6390	-0.7189	-0.7588	-0.8187	-0.8187	-0.1397	+1.6575
I207	0	-0.0079	-0.0479	-0.0799	-0.1439	-0.1439	-0.1439	-0.0479	+0.0479	+0.1919	+0.9119	+1.6639
I102	0	-0.0869		-0.1638		-0.2223		-0.3286				
I104	0	-0.0225				-0.0602		-0.1128		-0.1429		
I255								-0.3180				+1.1159
mean	-0.0582	-0.0857	-0.0725	-0.1556	-0.3019	-0.3179	-0.3396	-0.3933	-0.3319	-0.2131	+0.2371	+1.3216
1 S.E.	±0.0304	±0.0271	±0.0150	±0.0312	±0.1585	±0.0799	±0.1896	±0.0818	±0.2558	±0.1667	±0.3381	±0.2032

Appendix A.16 ΔK% responses to ISO. Perfusion line. Plus 5% serum.

conc. M	1×10^{-10}	1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
FISH NUMBER												
I201	-4.76	-6.34		-10.44		-21.64		-33.58				
I202	-4.14	-9.26		-11.67		-21.27		-33.28				
I203	-6.94	-5.45		-7.27		-20.33		-35.25				
I205	-5.85	-7.92	-7.92	-10.89	-16.33		-17.82	-23.04	-25.74	-25.74	-6.93	+97.02
I206	-3.06	-4.08	-5.10	-8.16	-31.63	-32.65	-36.67	-38.77	-41.83	-41.83	-7.14	+84.69
I207	0	-0.64	-3.84	-6.40	-11.53	-11.53	-11.53	-3.84	+3.84	+15.38	+17.03	+133.33
I102	0	-5.67		-10.72		-14.50		-21.44				
I104	0	-2.57				-6.85		-12.85		-16.28		
I255								-28.23				+45.77
mean	-3.09	-5.24	-5.62	-9.36	-19.83	-18.39	-22.01	-25.84	-21.36	-17.12	+19.65	+90.20
1 S.E.	±0.99	±0.98	±1.21	±0.77	±6.06	±3.17	±7.55	±3.43	±13.26	±12.05	±26.68	±18.05

Appendix A.17 ΔK responses (kPa $ml^{-1}min$) to ISO. Bolus. No serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles	1 μmole
FISH NUMBER										
I151	0			-0.2101		-0.2866	-0.2866	-0.3566		
I152	-0.0845		-0.0793	-0.1427	-0.1850					
I153	0		-0.0406	-0.1016		-0.1320	-0.0609	-0.1219		
I154							-0.4133			
I155		-0.0754	-0.0943	-0.1793			-0.2264	-0.2642	-0.2264	+0.0094
I156	-0.0079	-0.1592	-0.0159	-0.0239	-0.0358	-0.0597	-0.0637	-0.1623	-0.1745	+0.1195
mean	-0.0231	-0.0457	-0.0575	-0.1322	-0.1104	-0.1594	-0.2097	-0.2262	-0.2005	+0.0670
1 S.E.	±0.0205		±0.0179	±0.0324		±0.0669	±0.0675	±0.0527		

Appendix A.18 ΔK% responses to ISO. Bolus. No serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles	1 μmole
FISH NUMBER										
I151	0			-20.88		-30.61	-31.25	-38.88		
I152	-7.96		-8.06	-14.51	-18.81					
I153	0		-2.17	-5.43		-7.06	-3.39	-6.77		
I154							-27.41			
I155		-6.86	-8.58	-16.30			-20.60	-24.03	-20.60	+0.85
I156	-1.29	-2.58	-2.58	-3.89	-5.84	-9.74	-10.39	-16.88	-22.07	+19.48
mean	-2.29	-4.72	-5.35	-12.14	-12.32	-15.65	-18.60	-21.64	-21.33	+10.16
1 S.E.	±1.84		±1.72	±3.25		±7.44	±5.19	±6.74		

Appendix A.19 ΔK responses (kPa $ml^{-1}min$) to ISO. Bolus. Plus 5% serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
I251	-0.0768		-0.1344	-0.2876				-0.2977	-0.4707	
I252	0		-0.2593	-0.2593			-0.3649	-0.4369	-0.5378	
I253	-0.2176		-0.2418	-0.3748			-0.5321	-0.7074	-1.0280	-1.1852
I254	0		-0.0854	-0.1624			-0.7523	-0.8206	-1.0258	-1.0600
I255	-0.1940	-0.3638	-0.4245	-0.4614	-0.5458	-0.5822	-0.5822	-0.6186	-0.8126	-1.0431
I256	-0.3489	-0.1744	-0.4710	-0.6448	-0.9945	-1.1166	-1.1690	-1.1346	-1.3347	-1.3347
I257					-0.2599	-0.3198				
I258					-0.3447	-0.3447				
IB201	-0.2196	-0.1785	-0.3296	-0.3158	-0.3159		-0.3914	-0.3914		
IB202					-0.1663		-0.3327			
mean	-0.1509	-0.2389	-0.2780	-0.3580	-0.4378	-0.5909	-0.5893	-0.6296	-0.8683	-1.1557
1 S.E.	±0.0491	±0.0625	±0.0536	±0.0593	±0.1225	±0.1849	±0.1114	±0.1093	±0.1333	±0.0675

Appendix A.20 ΔK% responses to ISO. Bolus. Plus 5% serum.

Dose	10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
FISH NUMBER										
I251	-4.57		-7.99	-17.14				-17.77	-28.00	
I252	0		-14.09	-14.09			-19.83	-23.75	-29.23	
I253	-9.60		-10.66	-16.53			-23.46	-31.20	-45.33	-52.90
I254	0		-3.40	-6.46			-29.93	-38.77	-40.81	-42.17
I255	-7.96	-14.92	-17.41	-18.90	-22.38	-23.38	-23.88	-25.37	-33.33	-42.78
I256	-1.25	-6.26	-16.92	-23.19	-35.73	-40.12	-42.00	-40.75	-47.96	-47.96
I257					-18.26	-22.45				
I258					-19.99	-19.99				
IB201	-15.99	-12.99	-23.99	-22.99	-22.99		-28.49	-28.49		
IB202					-11.37		-22.74			
mean	-5.62	-11.39	-13.49	-17.04	-21.79	-26.48	-27.19	-29.44	-37.44	-46.45
1 S.E.	±2.25	±2.63	±2.57	±2.17	±3.06	±4.60	±2.79	±3.10	±3.45	±2.51

Appendix A.21 Mean peak response times (seconds) ± 1 S.E.M. for AD, NAD, ISO, administered by perfusion line.

conc. M		1×10^{-9}	5×10^{-9}	1×10^{-8}	5×10^{-8}	1×10^{-7}	5×10^{-7}	1×10^{-6}	5×10^{-6}	1×10^{-5}	1×10^{-4}	1×10^{-3}
DRUG	Serum/ No serum											
AD	No serum	349 ± 74	263 ± 57	236 ± 47	215	173 ± 34	160	145 ± 23	136	134 ± 9	98 ± 10	76 ± 7
AD	Serum	278 ± 52	241 ± 44	214 ± 31	187 ± 35	154 ± 19	145 ± 23	134 ± 29	145 ± 9	127 ± 8	90 ± 6	73 ± 7
NAD	No serum	473 ± 129	494 ± 94	395 ± 57	298 ± 52	240 ± 31	208 ± 21	123 ± 12	127 ± 16	117 ± 8	94 ± 10	91 ± 7
ISO	No serum	189 ± 20	191 ± 19	188 ± 16	223 ± 18	295 ± 14	271 ± 20	262 ± 34	231 ± 28	224 ± 23	690 ± 17	375 ± 16
ISO	Serum	184 ± 25	186 ± 29	212 ± 19	208 ± 19	237 ± 13	245 ± 24	271 ± 25	237 ± 41	229 ± 26	417 ± 50	356 ± 46

Values without standard errors are derived from two experiments only.

Appendix A.22 Mean peak response times (seconds) ± 1 S.E.M. for AD, NAD, and ISO administered as boli.

conc. M		10 pmoles	20 pmoles	50 pmoles	100 pmoles	200 pmoles	300 pmoles	500 pmoles	1 nmole	10 nmoles	100 nmoles
DRUG	Serum/ No serum										
AD	No serum		90	75 ± 15	69 ± 5	60 ± 3	63 ± 2	60 ± 1	60 ± 2	87 ± 9	75 ± 8
AD	Serum	75 ± 15	90 ± 15	81 ± 8	63 ± 4	62 ± 2	61 ± 1	58 ± 1	57 ± 2.4	55 ± 9	61 ± 10
NAD	No serum			150	120 ± 15	123 ± 2.4	102 ± 21	98.4 ± 14	96.6 ± 11.4	92 ± 11	104 ± 6
ISO	No serum	234	240	183 ± 33	245 ± 49	330 ±		224 ± 46	179 ± 28	224	225
ISO	Serum	240 ± 34	285 ± 26	297 ± 29	324 ± 50	309 ± 45		297 ± 37	193 ± 35	210 ± 26	231 ± 22

Values without standard errors are derived from two experiments only.

Appendix A.23 Responses to AD plus Phent given as a single bolus at dose ratios of 1 : 10 to 1 : 1000. From four preparations.

Dose moles		Initial control	1 : 10	1 : 25	1 : 30	1 : 40	1 : 50	1 : 100	1 : 200	1 : 1000	Final control
50p	ΔK	+0.0812		+0.0406			-0.0623	-0.0913		-0.152	+0.0408
AD	ΔK%	+4.73		+2.31			-3.46	-5.20		-8.67	+2.32
100p	ΔK	+0.0634	+0.0528	+0.0113	+0.0053	0		-0.711		-0.1726	+0.0451
AD	ΔK%	+8.32	+7.41	+1.48	+0.74	0		-4.04		-9.82%	+6.87
200p	ΔK	+0.583						-0.0729	-0.166		+0.255
AD	ΔK%	+53.33						-6.66	-13.37		+23.33

Appendix A.24 Mean responses to Phent control doses and concentrations (n = 3).

	Conc.	1×10^{-6} M	1×10^{-5}M	1×10^{-4}M
Response	ΔK	-0.0254	-0.0372	-0.0665
	ΔK%	-2.35	-4.08	-9.33

	Dose	10 nmoles	50 nmoles	100 nmoles
Response	ΔK	-0.0231	-0.0406	-0.1015
	ΔK%	-1.21	-2.31	-5.78

Appendix A.25 Responses to AD plus Phent administered via the perfusion line at concentration ratios of 1 : 10, 1 : 100 and 1 : 1000. From three preparations.

Conc.		Initial control	1 : 10	1 : 100	1 : 1000
1×10^{-8}M	ΔK	+0.187	+0.179	0.0	-0.137
AD	ΔK%	+20.42	+18.88	0.0	-9.79
1×10^{-7}M	ΔK	+0.522		-0.125	-0.077
AD	ΔK%	+20.97		-5.44	-5.77

Appendix A.26 Mean responses to ISO plus Prop administered as a single bolus at dose ratios of 1 : 1 to 1 : 20 (n = 2).

Dose	Dose ratio	Initial control	1 : 1	Control	1 : 5	Control	1 : 10	Control	1 : 20
200 pmoles ISO	ΔK	-0.316	-0.123	-0.233	-0.0822	-0.233	-0.0783	-0.315	-0.219
	ΔK%	-22.99	-6.04	-11.41	-3.61	-11.41	-3.37	-22.99	-9.64

Mean responses to ISO plus Prop administered as separate boli, Prop preceding ISO by 10 minutes at dose ratios of 1 : 1 to 1 : 20 (n = 2).

Dose	Dose ratio	Initial control	1 : 1	Control	1 : 2	Control	1 : 5	Control	1 : 20
200 pmoles ISO	ΔK	-0.734	-0.083	-0.734	-0.762	-0.730	-0.125	-0.721	+0.021
	ΔK%	-58.88	-5.47	-58.88	-4.76	-57.21	-4.79	-55.35	+1.67

Appendix A.27 Responses to ISO plus DCI administered as a single bolus, at dose ratios of 1 : 100 to 1 : 1000. From three preparations.

Dose	Dose ratio	Initial control	1 : 100	Control	1 : 200	Control	1 : 1000
500 pmoles	ΔK	-0.059	-0.048	-0.059	-0.048	-0.059	-0.048
	ΔK%	-9.74	-3.53	-9.74	-3.53	-9.74	-3.53
1 nmole	ΔK					-0.413	-0.194
	ΔK%					-27.41	-17.77

Appendix A.28 Mean responses to ISO plus DCI administered via the perfusion line at concentration ratios of 1 : 10 to 1 : 1000 (n = 3).

Dose		Control	1 : 10	Control	1 : 100	Control	1 : 1000
1×10^{-7}M	ΔK	-0.332	-0.022	-0.332	0.0	-0.597	+0.664
ISO	ΔK%	-20.33	-1.99	-20.33	0.0	-19.26	+30.05
1×10^{-6}M	ΔK	-0.575	-0.054	-0.575	+0.399	-0.619	+0.309
ISO	ΔK%	-35.25	-6.89	-35.25	+35.82	-40.10	+39.42

Appendix A.29 Mean responses to Prop and DCI control doses and concentrations.

	Dose	1 nmoles	5 nmoles	10 nmoles	20 nmoles	30 nmoles	50 nmoles	100 nmoles	
Prop	ΔK	-0.093	-0.141	-0.352	-0.189	-0.227	-0.589	-0.720	n = 3
	ΔK%	-4.13	-6.06	-15.15	-7.37	-8.81	-24.46	-31.72	
DCI	ΔK			-0.015				+0.055	n = 2
	ΔK%			-2.59				+9.09	

	Conc.	5×10^{-7}M	1×10^{-6}M	2.5×10^{-6}M	5×10^{-6}M	1×10^{-5}M	1×10^{-4}M	
Prop	ΔK	-0.152		-0.145	-0.208	-0.207		n = 4
	ΔK%	-7.73		-5.45	-9.69	-9.70		
DCI	ΔK		0.0			-0.044	+0.034	n = 2
	ΔK%		0.0			-3.98	+3.02	

Appendix A.30 Responses to ISO plus Phent administered via the perfusion line at concentration ratios of 1 : 10 and 1 : 1000. From two preparations.

Conc.	Initial control	1 :10	Control	1 : 1000	Final control
1×10^{-8}M	-0.0718	-0.0747	-0.0718	-0.0864	
ISO	-6.12	-7.01	-6.12	-7.36	
1×10^{-7}M	-0.222			-0.376	-0.194
ISO	-14.5			-26.79	-8.40

Appendix A.31 Responses to AD plus Prop administered as a single bolus at dose ratios of 1 : 5 to 1 : 100. From four preparations.

		Control	1 : 5	Control	1 : 10	Control	1 : 100
10 pmoles	ΔK	+0.103	+0.45			+0.266	+0.084
AD	$\Delta K\%$	+8.47	+5.22			+16.83	+3.34
50 pmoles	ΔK	+0.298	+0.193	+0.367	+0.823	+0.986	+0.211
AD	$\Delta K\%$	+23.24	+6.96	+22.49	+3.06	+71.68	+8.18
100 pmoles	ΔK	+0.734	+0.678	+0.301	+0.0823	+1.313	+0.344
AD	$\Delta K\%$	+59.88	+18.85	+18.33	+3.06	+94.29	+13.56
300 pmoles	ΔK					+1.820	+0.452
AD	$\Delta K\%$					+148.24	+16.09
500 pmoles	ΔK	+0.928	+0.715	+1.537	+0.941	+2.427	+0.522
AD	$\Delta K\%$	+75.70	+58.15	+93.33	+33.67	+173.14	+20.18
1 nmole	ΔK					+3.273	+0.625
AD	$\Delta K\%$					206.91	+26.143

Appendix A.32 Mean outflow changes (mls) ± 1 S.E. per minute of perfusion with AD.

n	conc. of AD M	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8
7	1×10^{-8}	0.8593	+0.0073	+0.0022	+0.0145	+0.0081	+0.0084	+0.0051		
	± 1 S.E.	±0.0667	±0.0099	±0.0037	±0.0087	±0.0045	±0.0173	±0.0056		
7	1×10^{-7}	0.8530	+0.0076	+0.0178	+0.0050	+0.0255	+0.0141	+0.0262	+0.0341	
	± 1 S.E.	±0.0746	±0.0072	±0.0051	±0.0194	±0.0064	±0.0103	±0.0043	±0.0072	
6	1×10^{-6}	0.8650	+0.0646	+0.0137	-0.0168	-0.0129	-0.0229	-0.0076	+0.0219	+0.0143
	± 1 S.E.	±0.0552	±0.0063	±0.0148	±0.0335	±0.0173	±0.0347	±0.0139	±0.0054	±0.0072
7	1×10^{-5}	0.8600	-0.0029	-0.0154	-0.0435	-0.0571	-0.0634	-0.0432	-0.0189	
	± 1 S.E.	±0.0578	±0.0109	±0.0238	±0.0234	±0.0164	±0.0263	±0.0100	±0.0315	
8	1×10^{-4}	0.8628	-0.0077	-0.0224	-0.0616	-0.0628	-0.0773	-0.0568	-0.0363	
	± 1 S.E.	±0.0573	±0.0112	±0.0170	±0.0202	±0.0242	±0.0282	±0.0229	±0.0031	
6	1×10^{-3}	0.8181	-0.0005	-0.0010	-0.0289	-0.246				
	± 1 S.E.	±0.0597	±0.0079	±0.0083	±0.0073	±0.0044				

Appendix A.33 Mean outflow changes (mls) ± 1 S.E. per minute of perfusion with ISO.

n	conc. of ISO M	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8	ΔOQ 8-9
5	1×10^{-9}	1.1282	-0.0071	-0.0065	-0.0211	+0.0046	-0.0139	-0.0158			
	± 1 S.E.	±0.0116	±0.0118	±0.0033	±0.0084	±0.0091	±0.0096	±0.0054			
6	1×10^{-8}	1.1128	+0.0000	-0.0110	-0.0107	-0.0296	-0.0068	-0.0093			
	± 1 S.E.	±0.0106	±0.0043	±0.0056	±0.0065	±0.0133	±0.0069	±0.0085			
6	1×10^{-7}	1.1163	-0.0056	0.00866	-0.0147	-0.0130	-0.0105	-0.0160	-0.0132		
	± 1 S.E.	±0.1085	±0.0095	±0.00864	±0.0140	±0.0083	±0.0109	±0.0105	±0.0054		
4	1×10^{-6}	1.1290	+0.0673	-0.01824	-0.0090	-0.0269	+0.0038	0	-0.0886	+0.0168	
	± 1 S.E.	±0.0954	±0.0073	±0.00851	±0.0065	±0.0074	±0.0051	0	±0.0021	±0.0073	
4	1×10^{-5}	1.0580	+0.0279	0.0	+0.0186	-0.0093	+0.0206	+0.0186	+0.0093	+0.0241	+0.0031
	± 1 S.E.	±0.1532	±0.0067	±0.0	±0.0251	±0.0089	±0.0153	±0.0094	±0.0031	±0.0201	±0.0097
4	1×10^{-4}	1.0141	+0.0149	+0.0052	+0.0021	+0.0035	+0.0100	+0.0053	+0.0071		
	± 1 S.E.	±0.1747	±0.0213	±0.0137	±0.0098	±0.0103	±0.0097	±0.0121	±0.0085		
4	1×10^{-3}	0.9028	0.0	+0.0179	0.0	+0.0171	-0.0030				
	± 1 S.E.	±0.0982	±0.0	±0.0106	±0.0	±0.0314	±0.0078				

Appendix A.34 Mean outflow changes (mls) ± 1 S.E. each minute after injection of AD.

n	Dose of AD	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8	ΔOQ 8-9	ΔOQ 9-10
17	Blank	0.7352	+0.07614	+0.00293	-0.00789							
	± 1 S.E.	±0.1257										
6	50 pmoles	0.76408	0.0845	0.01477	0.000245	-0.00144	+0.00148	-0.0110	-0.0125	-0.0135	-0.0171	-0.00336
	± 1 S.E.	±0.1009	±0.0196	±0.00953	±0.01533	±0.00613	±0.0205	±0.00524	±0.00648	±0.00751	±0.00973	±0.0249
6	100 pmoles	0.76002	+0.09012	+0.0191	+0.00196	-0.0078	-0.00464	+0.0054	-0.0114	-0.00223	-0.0037	-0.0101
	± 1 S.E.	±0.2038	±0.0146	±0.0122	±0.0178	±0.00764	±0.00877	±0.0161	±0.00480	±0.0162	±0.0052	±0.01129
5	200 pmoles	0.78991	+0.0905	+0.01292	-0.001302	-0.01089	-0.0047	-0.00457	-0.0079	-0.004	0.000	0.000
	± 1 S.E.	±0.20039	±0.00706	±0.01334	±0.01078	±0.01485	±0.00941	±0.00731	±0.00531	±0.00972	±0.000	±0.000
5	300 pmoles	0.79146	+0.09099	+0.00753	-0.00271	-0.0182	-0.00822	-0.00766	-0.0123	-0.00490	-0.0079	+0.0078
	± 1 S.E.	±0.2063	±0.01475	±0.00725	±0.1155	±0.0161	±0.01813	±0.00607	±0.00608	±0.0118	±0.00014	±0.00215

continued

Appendix A.34 (continued)

n	Dose of AD	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8	ΔOQ 8-9	ΔOQ 9-10
4	500 pmoles	0.8173	+0.01195	+0.00345	-0.01188	+0.00718	+0.00060	+0.00421	+0.0085	+0.0078		
	± 1 S.E.	±0.2187	±0.00463	±0.00959	±0.01172	±0.0101	±0.00085	±0.0067	±0.0095	±0.00667		
5	1 nmole	0.8255	+0.09166	+0.00189	-0.0162	-0.01474	-0.01004	-0.00517	-0.00743	-0.00212	0.000	
	± 1 S.E.	±0.2124	±0.0109	±0.02313	±0.01756	±0.0117	±0.01019	±0.00449	±0.00621	±0.00538	±0.000	
7	10 nmoles	0.7535	+0.09489	-0.0123	-0.0445	-0.0439	-0.03140	-0.02518	-0.01665	-0.0200	-0.02231	-0.02132
	± 1 S.E.	±0.1791	±0.0136	±0.02048	±0.0389	±0.02930	±0.02578	±0.02584	±0.02933	±0.01860	±0.0171	±0.02567
8	100 nmoles	0.7472	+0.07647	-0.04919	-0.08631	-0.10468	-0.09755	-0.09660	-0.09130	-0.08822	-0.09302	-0.0953
	± 1 S.E.	±0.1657	±0.02628	±0.03960	±0.05449	±0.05885	±0.05828	±0.04615	±0.04851	±0.04662	±0.04372	±0.05218

Appendix A.35 Mean outflow changes (mls) ± 1 S.E. each minute after injection of NAD.

n	Dose of NAD	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8	ΔOQ 8-9	ΔOQ 9-10
14	Blank	0.5557	+0.06589	-0.00330	-0.00015							
	± 1 S.E.	±0.0227	±0.006308	±0.008927	±0.00431							
7	500 pmoles	0.6079	+0.08487	+0.00686	+0.00460	-0.00503	-0.00230	-0.00955	-0.00501	-0.00278	+0.00583	+0.00070
	± 1 S.E.	±0.0393	±0.0112	±0.00451	±0.0130	±0.00497	±0.00472	±0.00449	±0.00599	±0.00460	±0.00505	±0.0080
7	1 nmole	0.6149	+0.09598	-0.00236	-0.0135	-0.0135	-0.01692	-0.01539	-0.0147	-0.00445	-0.00346	-0.0004
	± 1 S.E.	±0.00396	±0.0287	±0.00515	±0.0033	±0.00515	±0.00380	±0.00890	±0.00636	±0.00392	±0.00952	±0.00864
5	10 nmoles	0.61897	+0.08479	-0.0260	-0.0444	-0.0425	-0.0378	-0.0337	-0.0308	-0.0124	-0.00937	-0.01091
	± 1 S.E.	±0.04160	±0.00915	±0.0111	±0.0183	±0.0126	±0.01426	±0.0145	±0.0168	±0.00663	±0.00957	±0.00981
4	100 nmoles	0.6006	+0.09938	-0.0781	-0.2064	-0.23135	-0.22821	-0.21026	-0.19079	-0.1483	-0.1071	-0.09452
	± 1 S.E.	±0.0420	±0.0140	±0.0132	±0.0749	±0.1064	±0.1115	±0.1096	±0.10127	±0.09500	±0.06133	±0.04907

Appendix A.36 Mean outflow changes (mls) ± 1 S.E. each minute after injection of ISO.

n	Dose of ISO	Initial OQ	ΔOQ 0-1	ΔOQ 1-2	ΔOQ 2-3	ΔOQ 3-4	ΔOQ 4-5	ΔOQ 5-6	ΔOQ 6-7	ΔOQ 7-8	ΔOQ 8-9	ΔOQ 9-10
17	Blank	0.9186	+0.07876	+0.00124	-0.0008							
	± 1 S.E.	±0.1370	±0.01215	±0.0130	±0.0041							
3	20 pmoles	1.29105	+0.08609	+0.0144	+0.00006	-0.01065	+0.0104	+0.0144	+0.0152	0.0	+0.0013	+0.0072
	± 1 S.E.	±0.2051	±0.0139	±0.0097	±0.0084	±0.00937	±0.0138	±0.0138	±0.0139	0.0	±0.0159	±0.0136
4	500 pmoles	1.0945	+0.06909	+0.0088	-0.00895	-0.01145	-0.0238	-0.0105	+0.0072	-0.0105		
	± 1 S.E.	±0.1478	±0.0167	±0.00624	±0.0110	±0.0099	±0.1120	±0.0159	±0.0097	±0.0113		
5	100 pmoles	1.0556	+0.1017	-0.01317	-0.01351	-0.01449	-0.00408	-0.0232	-0.0311	-0.0216	0.0	
	± 1 S.E.	±0.1230	±0.0195	±0.0103	±0.0075	±0.01148	±0.00912	±0.0106	±0.00446	±0.0075	±0.0	
3	500 pmoles	0.9475	+0.0982	-0.00694	-0.00751	-0.1192	-0.0005	-0.00769	+0.0009	-0.0071	-0.0093	
	± 1 S.E.	±0.1560	±0.0165	±0.00342	±0.00393	±0.00179	±0.0074	±0.00629	±0.0073	±0.0059	±0.0103	
7	1 nmoles	0.9426	0.07998	-0.00854	-0.1993	-0.0155	-0.0103	-0.02061	-0.00676	-0.0093	+0.0072	-0.0071
	± 1 S.E.	±0.1462	±0.01413	±0.0118	±0.103	±0.00297	±0.0077	±0.01151	±0.0070	±0.00186	±0.00623	±0.0093
5	10 nmoles	1.0575	+0.05736	-0.0346	-0.02195	-0.01381	-0.01045	-0.0398	-0.03007	-0.0138	-0.0107	-0.0214
	± 1 S.E.	±0.1316	±0.00949	±0.0107	±0.00974	±0.00899	±0.00456	±0.00507	±0.01071	±0.00581	±0.0091	±0.01001
4	100 nmoles	1.0500	+0.09206	+0.0165	+0.01423	-0.01916	+0.0108	+0.0108	+0.0143	-0.0107	0.0	+0.0014
	± 1 S.E.	±0.1601	±0.0103	±0.00556	±0.00367	±0.0114	±0.00935	±0.00935	±0.0110	±0.0093	±0.0	±0.00713

Appendix A.37 Lymph heart frequency changes (ΔLHf) and amplitude (LHamp) during and after perfusion with whole eel blood.

No.	Initial LHf	Initial LHamp	ΔLHf 0-1	LHamp 0-1	ΔLHf 1-2	LHamp 1-2	ΔLHf 2-3	LHamp 2-3	ΔLHf 3-4	LHamp 3-4	ΔLHf 4-5	LHamp 4-5	ΔLHf 5-6	LHamp 5-6	ΔLHf 0-1	LHamp 0-1	ΔLHf 9-10	LHamp 9-10
N113	82	4.0	-2	4.5	+4	6.0	+4	5.5	+4	5.0	+4	4.5			+8	3.5	+6	2.5
A255	70	13.0	0	15.0	+2	14.0	+2	13.0	+6	12.0	+5	12.0			+7	9.0	+6	7.0
I106	82	3.5	+12	5.0	+10	8.0	+8	6.5	+7	5.5	+7	5.5	+7	5.5	0	5.5	+4	4.0
I107	72	7.0	+4	7.0	+10	12.0	+9	13.0	+12	13.0					+10	13.0	+2	7.0
I108	86	5.0	0	9.0	-1	10.0	0	9.5							-2	8.0	0	7.0

	WHOLE EEL BLOOD						RINGER	
FREQUENCY								
mean	78.4	+2.8	+5	+4.6	+7.25	+5.33	+4.6	+3.8
1 S.E.	±3.12	±2.49	±2.19	±1.71	±1.70	±0.88	±2.35	±1.28
AMPLITUDE								
mean	6.5	8.1	10	9.5	8.87	7.33	7.8	5.6
± 1 S.E.	±1.73	±1.90	±1.41	±1.57	±2.11	±2.34	±1.62	±0.88

Appendix A.38 Mean lymph heart frequency changes (ΔLHf) and lymph heart amplitude (LHamp) during infusion of AD.

n	Conc. of AD M	Initial LHf,	Amp	ΔLHf 0-1	LHamp	ΔLHf 1-2	LHamp	ΔLHf 2-3	LHamp	ΔLHf 3-4	LHamp
8	1×10^{-8}	62.12		-0.5		-0.25		+0.42		-0.28	
	± 1 S.E.	±3.86		±0.37		±0.35		±0.56		±0.37	
8	1×10^{-7}	63.87		-0.87		-0.75		-0.86		-1.28	
	± 1 S.E.	±4.00		±1.08		±0.79		±1.09		±0.94	
8	1×10^{-6}	67.2	2.3	+0.5	2.3	-1.25	2.56	+0.14	3.41	+0.28	4.75
	± 1 S.E.	±2.17	±0.54	±0.71	±0.54	±0.39	±0.88	±1.03	±0.51	±0.81	±1.09
8	1×10^{-5}	66.5	4.6	-0.25	4.7	+1.25	5.0	-0.37	5.45	+1.28	6.12
	± 1 S.E.	±4.07	±0.42	±0.87	±0.05	±0.39	±0.62	±0.77	±0.65	±0.69	±0.48
9	1×10^{-4}	68.2	3.12	0	3.37	7.55	4.25	3.0	5.07	3.57	4.32
	± 1 S.E.	±3.63	±0.77	±0.93	±0.79	±0.68	±0.73	±1.13	±0.71	±1.71	±0.89
5	1×10^{-3}	65.6		-1.0		+2.8		+9.0			
	± 1 S.E.	±7.06		±1.22		±0.73		±1.24			

continued

Appendix A.38 (continued)

n		ΔLHf	LHamp	ΔLHf	LHamp	ΔLHf	LHamp	ΔLHf	LHamp	ΔLHf	LHamp	ΔLHf	LHamp
		4-5		5-6		6-7		7-8		8-9		9-10	
8	1×10^{-8}	+0.20		-1.25		-0.66		0.0		0.0			
	± 1 S.E.	±0.96		±0.75		±1.04		±0.0		±0.0			
8	1×10^{-7}	-1.0		-1.0		-0.66		-0.50		-1.0			
	± 1 S.E.	±1.11		±1.21		±1.15		±1.25		±1.50			
8	1×10^{-6}	+2.0	5.12	+2.2	5.12	+4.74	4.75	+2.3	5.0	+12.5	5.0	+1.5	5.0
	± 1 S.E.	±0.86	±1.06	±1.02	±0.95	±1.49	±0.79	±1.91	±0.96	±0.78	±0.96	±1.39	±0.96
8	1×10^{-5}	1.28	6.87	2.66	7.43	+2.33	6.62						
	± 1 S.E.	±1.09	±0.32	±0.91	±0.38	±1.13	±0.55						
9	1×10^{-4}	+2.5	5.98	+7.5	6.0								
	± 1 S.E.	±1.69	±0.87	±1.08	±0.97								
5	1×10^{-3}												
	± 1 S.E.												

Appendix A.39 Mean lymph heart frequency changes (ΔLHf) during each minute after injection of AD.

n	Dose of AD	Initial LHf	ΔLHf 0-1	ΔLHf 1-2	ΔLHf 2-3	ΔLHf 3-4	ΔLHf 4-5	ΔLHf 5-6	ΔLHf 6-7	ΔLHf 7-8	ΔLHf 8-9	ΔLHf 9-10
4	1 nmole	78.25	-1.0	-0.25	-0.5	+0.25	+1.0	+1.5				
	± 1 S.E.	±1.84	±1.22	±0.63	±0.85	±0.29	±0.49	±0.85				
7	10 nmoles	55.0	-1.71	+0.14	+5.71	+9.14	+12.5	+15.0	+21.5	+23.66	+35.75	+35.75
	± 1 S.E.	±11.51	±2.85	±2.48	±3.05	±5.31	±6.94	±8.33	±9.36	±10.00	±10.65	±10.39
9	100 nmoles	68.33	-0.44	+3.66	+10.33	+13.88	+14.44	+13.66	+13.77	+14.33	+15.20	+14.16
	± 1 S.E.	±6.03	±0.33	±1.21	±2.47	±3.05	±3.97	±4.22	±4.59	±5.78	±6.57	±5.98

Appendix A.40 Mean lymph heart frequency changes (ΔLHf) and lymph heart amplitude each minute after injection of NAD.

n	Dose of NAD	Initial LHf	Initial LHamp	ΔLHf 0-1	LHamp 0-1	ΔLHf 1-2	LHamp 1-2	ΔLHf 2-3	LHamp 2-3	ΔLHf 3-4	LHamp 3-4	ΔLHf 4-5	LHamp 4-5
6	500 pmoles	40.83		0.0		+0.16		-1.0		-0.16		+0.08	
	± 1 S.E.	±5.25		±0.03		±1.02		±1.58		±1.43		±1.25	
7	1 nmole	35.57		-0.86		+0.14		-1.57		1.14		2.71	
	± 1 S.E.	±5.53		±0.51		±1.09		±1.04		±2.55		±2.97	
5	10 nmoles	41.01	2.35	+1.80	2.35	+3.45	2.37	+5.41	2.5	+5.41	3.0	+6.0	3.65
	± 1 S.E.	±3.02		±1.02		±2.30		±3.32		±3.29		±4.21	
6	100 nmoles	39.16	2.1	-0.16	2.35	+0.66	3.35	-0.16	3.6	+4.52	3.75	+8.83	3.5
	1 S.E.	±7.57		±1.64		±1.02		±1.71		±2.23		±4.11	

continued

Appendix A.40 (continued)

n	Dose of NAD	ΔLHf 5-6	LHamp 5-6	ΔLHf 6-7	LHamp 6-7	ΔLHf 7-8	LHamp 7-8	ΔLHf 8-9	LHamp 8-9	ΔLHf 9-10	LHamp 9-10
6	500 pmoles	-0.33		-0.40		+0.33		0.0		-0.5	
	± 1 S.E.	±0.87		±1.30		±0.28		±0.59		±0.45	
7	1 nmole	-0.14		0.57		-0.57		-1.33		-2.0	
	± 1 S.E.	±2.11		±2.42		±0.61		±0.99		±0.99	
5	10 nmoles	+4.8	3.75	+5.2	3.5	+5.2	4.0	+6.66	3.75	+4.66	4.0
	± 1 S.E.	±4.05		±3.87		±3.86		±4.93		±4.9	
6	100 nmoles	+9.83	3.56	+9.83	3.75	+10.8	4.0	+10.5	4.0	+11.5	4.0
	± 1 S.E.	±4.85		±4.87		±5.81		±6.19		±6.25	

Note. Standard errors for lymph heart amplitude are not given as only two preparations each at 10 and 100 nmoles gave sufficiently large records to accurately measure amplitude.

Appendix A.41 Mean values of cardiovascular parameters ± 1 S.E.M. during swimming at 15 cm s^{-1}. n = 8

	K_b kPa $ml^{-1}min$	Q ml min^{-1}	ΔP_g kPa	VAP kPa	DAP kPa	ΔP_t kPa	CVP kPa	Heart rate beats min^{-1}
rest	0.350 ± 0.071	6.95 ± 1.19	2.01 ± 0.203	5.15 ± 1.32	3.14 ± 0.146	2.14 ± 0.0987	0.989 ± 0.166	53.5 ± 1.07
5 min swimming	0.299 ± 0.0544	7.38 ± 1.22	1.79 ± 0.139	4.81 ± 0.115	3.02 ± 0.0776	2.08 ± 0.0823	0.889 ± 0.112	52.9 ± 1.06
15 min swimming	0.365 ± 0.0788	7.025 ± 1.23	2.07 ± 0.264	5.19 ± 2.00	3.14 ± 0.131	2.26 ± 0.186	0.881 ± 0.140	53.9 ± 0.953
30 min swimming	0.248 ± 0.0481	8.81 ± 1.52	1.98 ± 0.0596	5.13 ± 0.202	3.15 ± 0.0619	2.00 ± 0.198	1.14 ± 0.173	53.8 ± 1.56

Appendix A.42 Mean values of cardiovascular parameters ± 1 S.E.M. during swimming at 22-25 cm s^{-1}. n = 6.

	Kb kPa $ml^{-1}min$	Q ml min^{-1}	ΔPg kPa	VAP kPa	DAP kPa	ΔP_t kPa	CVP kPa	Heart rate beats min^{-1}
rest	0.322 ± 0.0405	6.79 ± 1.20	2.23 ± 0.300	5.26 ± 0.510	3.03 ± 0.223	2.23 ± 0.286	0.715 ± 0.0594	47.8 ± 2.21
5 min swimming	0.462 ± 0.0474*	6.36 ± 0.856	2.92 ± 0.616	5.99 ± 0.0544	3.07 ± 0.156	1.96 ± 0.257	0.884 ± 0.111	50.0 ± 2.32
15 min swimming	0.534 ± 0.0746*	6.43 ± 1.16	3.33 ± 0.609	6.24 ± 0.486	2.91 ± 0.202	2.079 ± 0.241	0.804 ± 0.614	49.2 ± 3.05
30 min swimming	0.568 ± 0.0717*	6.28 ± 1.03	3.60 ± 0.767	6.64 ± 0.569	3.04 ± 0.236	2.17 ± 0.227	0.912 ± 0.100	49.8 ± 2.18

* indicates significant change from resting value at $P < 0.05$ level. Paired Student's t-test.

Appendix A.43 Mean values of cardiovascular parameters ± 1 S.E.M. during recovery from swimming. n = 12

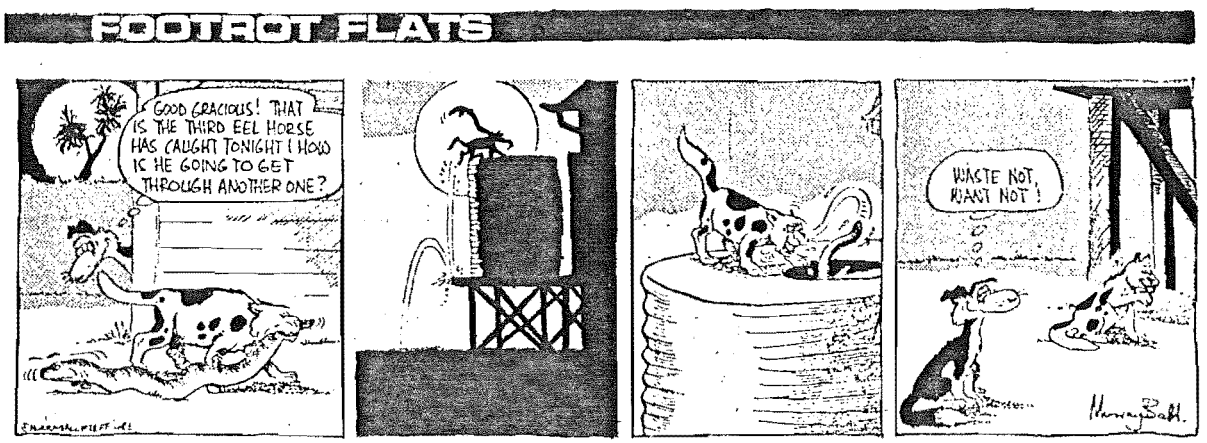
	Kb kPa $ml^{-1}min$	Q ml min^{-1}	ΔPg kPa	VAP kPa	DAP kPa	Δ P_t kPa	CVP kPa	Heart rate beats min^{-1}
End of swimming trial	0.555 ± 0.084	6.25 ± 0.831	2.96 ± 0.390	6.01 ± 0.335	3.06 ± 0.126	2.09 ± 0.100	0.950 ± 0.0985	48.2 ± 2.23
5 min rest	0.394 ± 0.071**	7.09 ± 0.987*	2.30 ± 0.333*	5.37 ± 0.278*	3.06 ± 0.125	2.14 ± 0.130	0.921 ± 0.107	50.5 ± 1.45
15 min rest	0.382 ± 0.0696**	7.18 ± 1.06*	2.25 ± 0.335*	5.33 ± 0.255*	3.08 ± 0.127	2.14 ± 0.130	0.887 ± 0.120	50.9 ± 1.49
30 min rest	0.351 ± 0.0706*	7.525 ± 1.075*	2.25 ± 0.423*	5.34 ± 0.313*	3.08 ± 0.127	2.33 ± 0.183	0.751 ± 0.164	50.0 ± 1.62

* indicates significant change from value at end of swimming trial at $P < 0.05$ level.

** indicates significant change from value at end of swimming trial at $P < 0.01$ level.

Paired Student's t-test.

Endpiece



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